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TROPICAL MEDICINE

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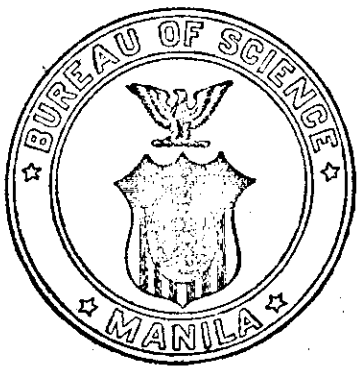

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THE PHILIPPINE
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B. TROPICAL MEDICINE

VOL. VIII DECEMBER, 1913 No. 6

BACTERIOLOGICAL OBSERVATIONS MADE DURING THE OUTBREAK OF PLAGUE IN MANILA IN 1912

By OTTO SCHÖBL

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

One plate

During the recent outbreak of plague in Manila, I had the opportunity to make certain observations which are of interest. These observations were made in the examination of: (1) Specimens taken from patients and from dead bodies at autopsies, (2) samples of bloodsucking insects collected in houses where plague patients had lived, (3) rodents caught by trap or poisoned in the parts of the city where plague cases occurred from time to time, and (4) domestic animals suspected of plague infection.

BACTERIOLOGICAL EXAMINATION OF PLAGUE PATIENTS

In order to secure as early diagnosis as possible, the following procedure of investigation was adopted:

1. The bubo was aspirated by means of a sterile hypodermic syringe. The material thus obtained was placed in the water of condensation of an agar-slant culture tube.

2. At least 7 centimeters of blood were withdrawn from the *cubital* vein by means of another sterile syringe, and 5 centimeters of it were placed in an Ehrlenmeyer's flask, containing 200 centimeters of neutral meat broth. The rest of the blood was emptied into a sterile tube, and used for agglutination tests.

Cultures obtained by this method were examined microscopically, and the growths on various culture media were studied. Gram stain, Löffler's methylene blue, and hanging-drop method were used. Polar-staining and chain formation in liquid media and the characteristic type of colony on the surface of agar were looked for. Animal inoculation was performed in every case, and the culture isolated from each case was identified by agglutination test, rabbit's immune serum being used.

The results of the bacteriological examination of a series of 24 patients are tabulated in the two following tables. Table I includes the fatal cases and Table II those cases which recovered.

TABLE I.—*Examination of fatal cases of plague.*

Patient.	Race.	Sex.	Age.	Date of examination.	Duration of illness.	Hours before death.	Bubo.			Blood.		Skin.			Sputum.		
							Smear.	Culture.	Animal inoculation.	Culture.	Agglutination.	Smear.	Culture.	Animal inoculation.	Smear.	Culture.	Animal inoculation.
			Years.	1912.	*Days.*												
1. Sing Nu	Chinese	Male	(?)	July 11	5	48	+	+	+	0	0	+	+	+	—	—	1 *
3. Asuncion Raymundo	Filipino	do	15	Sept. 29	3	.....	+	+	+	0	0	0	0	0	0	0	0
4. Filo Almalas	do	do	39	Oct. 10	4	22	+	+	+	+	-	+	+	+	0	0	0
6. Polycarpio Guzman	do	do	34	Oct. 22	2	.....	+	+	+	0	0	0	0	0	0	0	0
7. José Sarmiento	do	do	37	do	3	.....	+	+	+	0	0	0	0	0	0	0	0
8. Julian Gonzales	do	do	41	do	3	23½	0	0	0	+	—	0	0	0	0	+	0
9. Valeriano Buencamino	do	do	31	do	3	10	+	+	+	+	—	0	0	0	0	0	0
10. Pedro Nicomedes	do	do	30	do	2	5½	+	+	+	±	—	0	0	0	0	0	0
12. Regino Gulano	do	do	34	do	2	106	0	0	0	±	—	0	0	0	0	0	0
				Oct. 24	4	82	0	0	0	0	0	0	0	0	0	+	+
13. Martin Dimalanta	do	do	35	Oct. 23	3	25½	±	+	+	+	—	0	0	0	0	0	0
14. Roberto Obiso	do	do	25	do	1	53	+	+	+	+	—	0	0	0	0	0	0
15. Juan Barceta	do	do	23	Oct. 24	3	37	+	+	+	+	—	0	0	0	0	0	0
16. Yu Tum	Chinese	do	14	do	2	.....	±	+	+	0	0	0	0	0	0	0	0
17. Augustin Monterey	Filipino	do	29	Nov. 1	1	27	+	+	+	+	—	0	0	0	0	0	0
18. Demetrio Pabraw	do	do	27	Nov. 23	4	15	0	0	0	+	—	+	+	+	0	0	0
21. Ambrosio Sobremonte	do	do	20	Dec. 7	6	1	±	+	±	+	—	0	0	0	0	0	0
22. Mateo Marcelo	do	do	8	Aug. 20	(?)	.....	—	—	—	0	0	0	0	0	0	0	0
23. Alejandro Gita	do	do	*17	Nov. 24	3	.....	—	—	—	0	0	0	0	0	0	0	0

* Months.

TABLE II.—*Examinaton of plague patients who recovered.*

Patient.	Race.	Sex.	Age.	Date of examination.	Duration of disease.	Bubo.			Blood.	
						Smear.	Culture.	Animal inoculation.	Culture.	Agglutination.
			Years.	1912.	*Days.*					
				Sept. 29	2	—	—	—	0	0
				Oct. 2	5	+	+	+	0	0
2. Dionisio Capate	Filipino	Male	18	Oct. 3	6	0	0	0	—	+1:16
				Oct. 7	10	—	—	—	0	0
				Oct. 15	18	—	—	—	—	+1:64
5. Alejandra Fisher.	European	Female	6	Oct. 20	7	+	+	+	0	0
				Oct. 22	2	+	+	+	+	—
				Oct. 24	4	+	+	+	0	0
11. Gabriel Sevilla	Filipino	Male	21	Oct. 26	6	0	0	0	—	+1:16
				Nov. 8	18	—	—	—	0	0
				Nov. 15	25	—	—	—	—	+1:64
				Nov. 26	3	+	+	+	+	—
				Dec. 6	13	0	0	0	—	+1:32
19. Esteban Roa	do	do	15	Dec. 16	23	—	—	—	—	+1:60
				1913.						
				Jan. 11	48	—	—	—	—	1:120
				Dec. 2	(?)	+	+	+	0	0
20. Sia Su	Chinese	do	35	Dec. 5	—	0	0	0	—	—
				Dec. 16	—	—	—	—	—	+1:80
24. Purificacion del Val.	Filipino	Female	19	Dec. 11	3	+	+	+	0	0
				Feb. 11	33	—	—	—	0	0

NOTE.—The bubo in Nos. 2, 5, and 24 never opened spontaneously. The pus was aspirated at the time of the second, eventually third, examination. Nos. 11 and 19 opened spontaneously. A fistula formed along the canal which was caused by the puncture, and healed up in several weeks. Hard inguinal buboes of secondary order persisted in patient 19 at the time of second examination. No plague bacilli were found either in the bubo of the first or second order. Patient 20 had a considerable amount of pus in the inguinal primary bubo, but it was not opened until after the last examination.

The diagnosis of plague could be safely made from the microscopical examination of the liquid aspirated from the bubo in the majority of the cases. However, in certain instances the amount of the aspirated fluid being small and the bacilli very few, it was impossible to diagnose the case, especially when the cultures from the bubo were negative. Repeated examination of the patient was necessary under those conditions, but it happened in cases 22 and 23 that the patients died of plague before a second examination could be made. The smears and cultures from case 22 remained sterile, while the smears and cultures made from the swelling on the neck of patient 23 revealed the presence of pneumococci. Both patients died of

plague, as was ascertained by examination of the organs after death.

Two of the patients, cases 8 and 12, had numerous plague bacilli in the sputum at the time when the expectoration showed the presence of blood (twenty-three and one-half and eighty-two hours, respectively, before death). In 3 cases I was able to prove the presence of *Bacillus pestis* in the skin lesions, *intra vitam*, fifteen, twenty-two, and forty-eight hours, respectively, before death. In case 18 there was no doubt that the skin lesions, which covered the whole body and the face, were of secondary nature, as the patient died shortly afterward. It was undoubtedly a case similar to those reported by Gotschlich and Zabolotny.[1] In the other two patients there was only 1 maculopapulous efflorescence on the foot in case 1 (with a corresponding femoral bubo) and 2 lesions of the same type on the arm and forearm in case 4 (with a corresponding axillary bubo). It is possible that these lesions were the original port of entry of infection. Numerous plague bacilli were found in the skin lesions of these cases, both microscopically and in culture.

The plague patients tabulated in Table II recovered. They were all treated with antiplague serum. While cases 5, 2, 19, and 24 appeared clinically to be rather severe, cases 2 and 20 were mild.

It can be seen from the table that the plague bacilli may not be detected in the enlarged gland at first (case 2) and that their presence may be revealed only after repeated examination of the bubo. It is also evident from the results of repeated examinations that the plague bacilli disappear from the infected gland in a comparatively short time, as a rule at the time when pus starts to form. Contrary to the findings in patients who died, distinct phagocytosis was noticed in the smears made from the aspirated liquid in those patients who recovered and who had been treated with serum soon after the onset of the disease. It is undoubtedly this process that clears the gland of the infectious agents.

The general opinion in regard to the presence of *Bacillus pestis* in the circulating blood seems to have been, as Thompson remarks, that "the bacillus is rarely to be found in the peripheral blood stream before the agonal stage."[2]

The Austrian Commission, using few drops of blood, found

[1] Kolle und Wassermann. Handbuch der pathogenen Mikroorganismen. Gustav Fischer, Jena (1903) 2, 521.

[2] *Journ. Hyg.*, Cambridge (1906), 6, 558.

positive blood culture in 40 per cent; Calvert in Manila in 100 per cent when examined twenty-four hours before death; Choksy, Berestneff, and Mayr in 45 per cent; and Greig in 60 per cent. The Indian Commission examined 28 patients, and obtained positive blood cultures in 16 out of 23 fatal cases. Not a single positive blood culture was obtained from the patients who survived. The time of blood examination in positive cases was three and one-half to seventy-five and one-half hours before death. The amount of blood used was 1 cubic centimeter. Only 6 out of the 30 samples, which gave positive blood culture, were found positive by microscopical examination of blood smears. The following conclusions are based on these observations in regard to the septicæmic stage of bubonic plague: (1) "A severe septicæmia may be present at a comparatively early stage of the disease and for a considerable number of hours before death, and (2) the septicæmia may be of an irregular and fluctuating type." [3]

From the tables it will be seen that out of 15 patients examined by me, 14 gave positive blood culture; and of these 3 recovered. One blood culture revealed the presence of streptococcus in addition to *Bacillus pestis.* The results of the examinations tabulated in Tables I and II show, in agreement with the findings of the Indian Commission, the occasional early occurrence of plague bacilli in the blood stream, as the time of examination in the positive cases varied from one hour to one hundred six hours before death. In consideration of the ephemeral character of the septicæmic stage of plague, as evidenced by repeated blood cultures in the three patients who recovered, one can hardly avoid the impression that there is a certain degree of septicæmia in every case of plague. The possibility of detecting the bacillus in the circulating blood increases in proportion with the quantity of blood used for culture. The best chance to recover plague bacilli from the circulating blood seems to be in the stage of high fever and general prostration.

The phenomenon of agglutination of plague bacilli by the serum of patients was first observed by Wissokowitsch and Zabolotny in 1897 [4] and later confirmed by the German Plague Commission. Vagedes, Klein, and others [4] pointed out the defects of the reaction as a diagnostic means. Aside from the technical difficulties, the reaction was found inconstant, and its

[3] *Ibid.* (1907), 7, 395.

[4] Referred to in Kolle und Wassermann. Handbuch der pathogenen Mikroorganismen (1903), 2, 524.

transmission of plague. Although many investigators have been successful in demonstrating the presence of *Bacillus pestis* in the digestive system of bloodsucking insects, it was not until the experiments of Gauthier and Raybaud that the actual transmission of plague infection by fleas was convincingly proved. Ever since the exhaustive and conclusive experiments, which were carried out both under natural and artificial conditions by the British Plague Commission, and the work of Verbijtski, which antedates the British Commission, were presented, there has been no doubt that the transmission of plague by bloodsucking insects particularly by the fleas is one, although not the only, mode of spreading this disease. It is obvious, as Herzog correctly remarks, that the factors which are responsible for the spreading of plague must be considered individually in each epidemic and in various parts of the world as well. There is no doubt that the importance of any insect in the transmission of plague depends on its habits as well as on those of the host, be it either animal or man.

TABLE III.—*Insects found to contain Bacillus pestis.*

Author.	Insect.	Source of infection.	Experimental transmission.
Yersin	Flies	Laboratory infection	
Nuttal	do	Experimental infection	
Do	Bedbugs	do	Negative by bite.
Do	Flea	do	Negative.
Hankin	Ant's fæces	Fed on plague material	
Do	Bedbugs	Plague hospital	
Ogata	Flea	Plague rats	
Simond	do	Plague rats, experimental	Positive.
Tindswell, 1900	do	Plague rats	Negative.
Tindswell, 1903	do	do	Do.
Kolle	do	Experimental infection	Do.
Gauthier and Raybaud	do	do	Positive.
Liston	do	Epidemic among pigs; harbored fleas; dead rats found.	Do.
Zirolia	do	Retained *Bacillus pestis*, 7-8 days	
British Commission	do	Repeated experiments	Do.
Verbijtski	Flea and bedbug	Experimental infection	Do.
La Bonadière and Xanthopulides.	Fly		
Herzog	*Pediculus capitis*	Dead body of a plague case	

During the recent outbreak of plague in Manila, several samples of bedbugs from the beds of the plague patients and dog fleas from a plague-infected house were collected and examined, but with negative result.

In spite of the fact that it adds nothing new to the question

of whether or not plague can be transmitted by fleas, since the question has been conclusively answered by the work of the Indian Commission, nevertheless the following observations of a small outbreak of plague among animals, the spreading of which was due solely to fleas, are of interest.

One wild rat was inoculated with strain Iloilo 3 of *Bacillus pestis*. The skin adjoining the root of the right ear was scarified, and a loopful of the culture was smeared on the scarified skin. The rat was found dead three days after the inoculation.

The cage containing the dead rat was immersed in kreolin solution. At autopsy the cervical glands were found slightly swollen, somewhat reddened, but no hæmorrhagic œdema of the surrounding tissue was noticeable. There was slight necrosis at the place of inoculation, showing superficial, purulent discharge. Clear effusion in both pleural cavities and one hæmorrhage in the pleura were found. The lungs were hyperæmic, but otherwise normal. The spleen was of somewhat darker color, but otherwise normal in size and appearance. The liver showed a slight degree of parenchymatous degeneration, the congestion making prominent the structure of the organ. The typical, although not constant, changes of the organ, which are characteristic of natural plague infection in rats, were absent. The kidneys were without macroscopic change. The lymph glands, with exception of the cervical nodes, were normal.

Examination of the rat's fur revealed ectoparasites on the neck, under the chin, and back of the ears; these at the time of the examination apparently were dead. About 6 common rat fleas were found and identified as *Lœmopsylla cheopis* Rothsch. The parasites were immersed in sterile salt solution for three hours. When removed in a dry test tube, they began to move about sluggishly. The intestinal tract of these fleas contained blood.

Five of the fleas were crushed by means of sterile forceps, and inserted in a pocket under the shaved skin of a guinea pig. The animal died of plague within three days, showing considerable hæmorrhagic œdema around the place of inoculation, typical bilateral inguinal buboes, and characteristic changes in the spleen. Smears and cultures made from the bubo and spleen were positive for *Bacillus pestis*.

Another wild rat, which was in a separate cage in the same room where rat 1 had been kept, died twenty-four hours after rat 1. The two cages were at least 10 centimeters apart. Rat 2 harbored fleas of the same species as were found on rat 1.

Numerous severe bites were detected back of the ears and on the neck of the dead animal. The post-mortem findings were identical with those described in rat 1; that is, cervical buboes, pleural effusion, and slightly enlarged spleen.

It is well to remark that both rats had been kept in the same room for about six months. Fleas had never been noticed on our guinea pigs. During the time the rats had been kept in the plague house no irregular results were noticed in plague-inoculated animals. At the time the first rat was inoculated no other plague-infected animals were in the plague house, and since that time another building has been used for plague-infected animals.

The attached plan of the plague house shows the location and time of death of these and the other animals in this outbreak.

Two days after the death of rat 2 three guinea pigs, which were kept in separate cages in the same room, were found dead of plague (smears and cultures were both positive). Several fleas (*Lœmopsylla cheopis*) were found on the necks of these animals. They were collected and inoculated in the same way as the fleas from the first rat. The experimental animal, which was inoculated with the fleas, was killed and found to be infected with plague. The findings were local reaction, inguinal buboes, and typical spleen. Smears and cultures were positive for *Bacillus pestis*.

Although numerous healthy guinea pigs were examined in the same plague house, no fleas could be found at that time, only the 2 rats and the first 3 guinea pigs are positively known to have harbored fleas, the latter after the death of the rats and not before.

The gross lesions in these naturally infected guinea pigs were somewhat unlike those found in guinea pigs infected either by vaccination or by intraperitoneal or subcutaneous inoculation. All except one showed primary buboes on the neck with more or less extensive hæmorrhagic œdema extending in some cases over the thorax. There was little pleural effusion present; the spleen always showed typical changes of necrotic foci varying in size and number. In one instance similar foci were found also in the liver, large enough to be visible macroscopically. This was in a case where like changes were found in the lungs.

Only one of the guinea pigs showed an exception, in that the primary buboes were located in the inguinal region, with pelvic and axillary glands secondarily involved. These are the findings usually met with in guinea pigs artificially infected with plague

by the vaccination method, if the lower part of the abdomen be chosen for inoculation. The reason for such a deviation from the findings in the rest of the guinea pigs may lie in the fact that this animal was almost completely deprived of hair by a skin disease.

It is of importance to mention the skin lesions which were found on the necks of the guinea pigs, particularly under the chin. Besides small red spots which appeared to be fresh flea bites, small, elevated, and fairly deep infiltrations partly covered with moist scab were found in the skin under the chin. Other animals showed changes usually found in the scarified skin of guinea pigs after artificial inoculation with plague material. The base of each cutaneous efflorescence was hæmorrhagic and œdematous.

A histological study of the tissues of these guinea pigs known to be naturally infected by plague fleas showed the following changes:

The cervical bubo.—The enlarged lymphatic gland was surrounded with a thickened capsule. Necrosis existed in the subcapsular part of the gland, where it formed an almost continuous circular zone, leaving the central part less changed. Smaller irregular necrotic foci were scattered throughout the section. Polymorphonuclears in various stages of disintegration were found throughout the section.

The lungs.—Very few blood extravasations were present in the alveoli; otherwise normal.

The spleen.—The capsule was thin. There were subcapsular hæmorrhages. The Malpighian bodies were somewhat enlarged, but of normal structure. Throughout the parenchyma irregular multiple necrotic foci were found, leaving but little of spleen tissue intact. Numerous polymorphonuclears which were present showed varying degrees of karyorrhexis.

The kidneys.—The outline of the cells was indefinite; a few miliary hæmorrhages existed in the cortical part of the organ.

The liver.—There was excessive congestion, fatty degeneration, and pigmentation of the cells. The capsule was slightly thickened.

The skin.—The epithelium was missing in one place in the section, and cellular infiltration extended from that place into the subepithelial layer of the surrounding skin. The same kind of infiltration reached deep into the skin, stripes of cellular infiltration penetrating into the tissue along the muscle fibers. There was no direct connection between the cellular infiltration and the follicles of the hair.

It may be well to describe in detail the time of death from plague among these and the other animals in this outbreak, as well as the time when the plague house was disinfected.

The first animal (rat 1) having been inoculated on August 27, in the afternoon, died of plague within three days (August 30). The second animal (rat 2) died twenty-four hours later.

Guinea pigs 3, 4, and 5 (see plan) were found dead on the morning of September 2; that is, two days after the death of rat 2 and three days after the death of rat 1.

The same day that the three guinea pigs were found dead of plague, rooms I, III, IV, and VI (see plan) were thoroughly disinfected. The floor, the ceiling, and the walls were sprayed with kerosene and lysol solution. The remaining animals in room VI were destroyed, and the cages disinfected. No animals were kept in rooms I, III, and IV at that time.

Three days after the death of animal 5, guinea pigs 6 and 7 were found dead of plague, while the next day guinea pigs 8 and 9 died. No death occurred on September 7, but the next two days each recorded two plague guinea pigs (10, 11, 12, and 13). On September 11, the last guinea pig died of plague in this outbreak. The whole building was then thoroughly disinfected. No plague-inoculated animals were kept in the rooms after the first sign of the epidemic. After September 11, no more cases of spontaneous plague infection were observed.

It will be noticed that the epidemic lasted eleven days after the first animal died and fourteen days after animal 1 was inoculated. Altogether, 14 animals out of at least 200 animals exposed died of plague.

No death occurred among rabbits, although these animals were distributed among the guinea pigs. In fact, 2 rabbits were surrounded by plague guinea pigs 8, 9, and 10 (see plan), but did not contract plague.

From the epidemiological standpoint it is interesting to know the dimensions and location of the cages in which the animals were kept.

Aside from the 2 rats which were confined in ordinary traps that stood on a table 80 centimeters high, the rest of the animals were kept in regular metal animal cages. The dimensions of the cages are: Fifty centimeters long, 36 centimeters broad, and 30 centimeters high. The cage stands on four legs each 10 centimeters long; the center of the bottom of the cage holds a drain opening 8 centimeters above the floor.

The majority of the cages in room II were located on the floor; some on the second shelf of a wooden rack. This last-mentioned arrangement, judging from the construction of the wooden frame, allowed a continuous passageway for the fleas to the second shelf of the racks. On the other hand, the deaths among the guinea pigs in room V were restricted to the cages standing on the floor, the majority of cages in that room being placed on tables 80 centimeters high.

Only a theoretical explanation can be given of the short duration and sudden cessation of the outbreak. One can assume with great probability that the first partial disinfection drove the fleas away from the primary source of infection, and that they traveled as far as possible. They finally settled in those guinea-pig cages which had not been molested by the first disinfection. Having no new supply of plague blood (all of the plague-infected guinea pigs having been removed, most of them before death), the fleas soon cleared themselves of plague bacilli. The peculiar feature of the outbreak; namely the failure to find fleas on the animals in rooms II and V, finds its explanation in the observation of the Indian Commission who found that the fleas "died or disappeared very rapidly."

The following conclusions can be drawn from these observations:

1. The common rat flea (*Lœmopsylla cheopis*) prefers the rat to the guinea pig.

2. In the absence of rats it will attack guinea pigs rather than rabbits.

3. The fleas which have sucked blood from rats or guinea pigs afflicted with plague septicæmia were found to harbor virulent plague bacilli inside of their bodies.

4. The transmission of plague infection by direct or indirect contact being excluded in our case, the fact that fleas of the same species and harboring plague bacilli were found on the rat and on the guinea pigs, the presence of flea bites on the rats and on the guinea pigs with positive findings of skin lesions on that part of the body where the fleas and flea bites were located, together with the anatomical picture of the findings in the guinea pigs, lead to but one explanation; namely, that the plague infection was transmitted by fleas.

OBSERVATIONS ON ANIMALS SUSPECTED OF PLAGUE

Out of the several tens of thousands of rodents examined during the antirat campaign, we have found only two plague rats which showed the typical picture of natural plague infection in rat; that is, cervical buboes with surrounding œdema, subcutaneous injection, pleural effusion, enlarged spleen, and such changes of the liver as are characteristic of natural plague infection in rats. Microscopically, large numbers of plague bacilli were found in these cases, and pure cultures of *Bacillus pestis* were recovered from the spleen. Histological examination of internal organs, particularly that of the liver, confirmed the bacteriological findings. The remainder of the plague rats

exhibited only two of the signs of plague infection; namely, bubo and œdema of the surrounding tissue, and eventual hæmorrhages.

Besides plague infection, a great number of rats showed purulent conditions from causes other than plague. Abscesses of the lungs were frequently met with, and cervical or axillary buboes are not uncommon in Manila rats. Various pyogenic bacteria were found in the pus of such abscesses. Of the less common was *Bacillus pyocyaneus* and the pneumobacillus of Friedländer. Chronic plague was excluded in these cases since the animal inoculation failed to produce plague infection.

More than half of the rats examined harbored parasites in their organs. *Echinococcus teniæformis* was found in the liver of practically every gray rat, while a small *Ascaris* and *Tænia diminuta* were not uncommon in the intestines. Two rats were found to have sarcosporidiosis, 2.6 per cent showed rat leprosy, and 7.4 per cent trypanosomiasis. One tumor of the mammary gland and one tumor in the axillary region were encountered, while one tumor of the large curvature of the stomach proved to be a chronic inflammatory tumor due to parasites. One peritoneal tumor in a rat (*Mus decumanus*) gave the impression of a malignant tumor on account of the miliary dissemination in the peritoneum. It was found to consist of muscle and spindel-cell sarcomatous tissue. Ectoparasites were very seldom noticed, on account of the method of collecting the rats. When present, they were mites and fleas.

In the naturally infected plague rats the rigidity of the fresh cadaver was pronounced. The primary bubo was in every case cervical. Cervical glands were enlarged and hæmorrhagic with slight œdema of the surrounding tissue. The subcutaneous injection extended over the neck and chest. The inguinal glands were small and pigmented. The lungs were collapsed, and showed hæmorrhagic foci. The spleen was slightly enlarged, firm, and dark red. The liver was rather large, firm, pale red, with shade of yellow, which was caused by minute yellowish foci thickly scattered throughout the tissue and visible through the capsule. The kidneys were hyperæmic. The intestines were without change. The serous membranes were pale with no hæmorrhages.

Histological examination of the tissue of naturally infected plague rats showed the following changes:

Liver.—The structure of the organ was well marked; the veins dilated, trabeculæ slightly compressed, nuclei well stained, and few of the liver

cells showed vacuoles. Small foci, most numerous under Glisson's capsule, were scattered throughout the organ; they varied in size, but were not larger than a miliary tubercle. The small necrotic foci were found to consist of few necrotic liver cells. The center of the larger foci was formed by degenerated and necrotic liver tissue, surrounded by round-cell infiltration. Polymorphonuclears were also found in the zone of cellular infiltration. There was a slight degree of hæmorrhage in each focus. Epitheloid cells and large vesicular cells with several nuclei were to be found. The foci, mentioned above, were sharply demarcated from the surrounding liver tissue, which appeared to be intact.

Spleen.—The structure was well preserved, the capsule thin. The Malpighian bodies were normal as to the elements of which they consist. Cells with pycnotic nuclei were scattered throughout the organ, and vesicular cells with small, deeply stained, excentrically located nuclei were present. Polymorphonuclears were found in the tissue in considerable numbers. No localized necrotic foci could be found in sections through the spleen.

Cervical glands.—The blood vessels were considerably distended. A few hæmorrhages and polymorphonuclears were present. Œdema of the capsules and surrounding tissue existed. Part of the gland was necrotic.

Lungs.—The blood vessels were distended. The alveoli contained homogeneous masses and blood. There were numerous subpleural hæmorrhages. The bronchi were collapsed, and contained mucus.

Kidneys.—The cortical part showed subdued structure; the epithelial cells had an indefinite outline and occasionally showed vacuolization. The medular part was better preserved. There were miliary subcapsular hæmorrhages. A few small foci were scattered throughout both medular and cortical parts. They consisted of round-cell infiltration.

NATURAL PLAGUE INFECTION IN A CAT

The experiments of the German Plague Commission proved that cats showed considerable resistance to plague infection as cutaneous and subcutaneous inoculations failed to infect them. According to the Austrian Commission, cats develop submaxillary buboes if fed on plague material. They are said by Albrecht and Gohn [9] sometimes to recover. Out of four cats fed on plague material two died of plague, one showing submaxillary the other mesenterial buboes. Virulent plague bacilli were found in the discharge from the nose and also in the fæces of cats which apparently did not become infected after having been fed on plague material.

One case of spontaneous plague infection of a cat was recorded by Thompson [10] in Sydney.

W. Hunter [11] in Hongkong made observations on cats suffer-

[9] Über die Beulenpest in Bombay im Jahre 1897. (1897), II B, II C.

[10] Report of an outbreak in Sydney, 1900. Referred to in Kolle and Wassermann (1903), 2, 510.

[11] *Lancet* (1905), I, 1064.

ing from plague infection. The author also undertook a few experiments, and arrived at the following conclusions:

1. Cats suffer from plague.
2. The disease may be acute or chronic.
3. The type of the disease is septicæmic.
4. The animals may occasionally play a part in the dissemination of plague.
5. In plague-infected areas cats probably become infected through rats, which they devour as food.
6. In plague-infected districts possible plague infection in cats is of great importance from a domestic point of view.

On November 27, 1912, a sick cat was brought to the laboratory for examination. It was reported that the animal was found in a warehouse in which dead rats had been found some time previously. The rats were not examined. In the morning of the 30th, the cat was found dead in the cage where it had been kept under observation. The following are the post-mortem findings:

The animal was a fairly well-nourished female.[12] The subcutaneous tissue, pericardium, mediastinum, and mesenterium contained considerable amounts of fat.

The subcutaneous tissue of the neck showed œdema and small hæmorrhages. The submaxillary tissues were swollen on both sides. When the fasciæ and superficial muscles of the neck were removed, enlarged glands were found on both sides. These were closely attached to the submaxillary salivary glands. The surrounding tissue was œdematous, but no hæmorrhages were noticed in the vicinity of the enlarged glands. Upon section the glands were found to be necrotic, and upon pressure a thin purulent liquid escaped. There were no hæmorrhages within the glands. Several enlarged lymph nodes, smaller in size, could be followed down the neck on the left side. The lymph nodes in the axillæ as well as in the groins and peribronchial nodes were normal. The mesenteric glands were slightly enlarged and reddened.

The lungs were slightly collapsed. A clear, sanguinous, slightly coagulated effusion was observed in both pleural cavities. The tissue of the lungs showed considerable œdema and hypostasis. The bronchi and pharynx showed no changes, the mucous membrane being pale and thin.

The heart was normal.

The spleen was enlarged, of light red color, with follicles slightly prominent.

The stomach contents was blackish in color; there were no hæmorrhages or ulcers in the mucosa.

[12] The cat was the mother of 4 kittens which were about 3 weeks old at the time the cat was delivered for examination. They were kept under observation for several weeks, but showed no signs of plague infection.

The liver was somewhat enlarged. The organ showed prominent structure, the centers of the acini being red, the periphery lighter in color.

The kidneys were slightly enlarged and pale. The capsule peeled off easily, the venæ stellatæ were prominent, the surface smooth; there were no hæmorrhages. The cortex was increased in breadth and was of the same color as the surface; the pyramids were darker in color. The organ was of fragile consistence.

Suprarenals were normal, as were also intestine and bladder.

The histological findings were as follows:

Bubo.—The capsule of the gland was œdematous. The whole gland as seen in cross section had undergone necrosis, except a few foci which still showed cellular structure.

Lungs.—The alveoli were filled with homogeneous masses, containing but few degenerated epithelial cells and leucocytes. The blood vessels were dilated, particularly in the subpleural part of the organ. In some places capillary mycotic emboli with subsequent hæmorrhage were encountered. The large blood vessels and bronchi were normal.

Salivary gland.—Those glands attached to the primary bubo showed the normal structure of a combined mucous and serous gland.

Liver.—There was considerable congestion. The centers of the acini showed parenchymatous and fatty degeneration. The cells on the periphery of the acini exhibited typical fatty infiltration. The large blood vessels and small ducts were without change.

Kidney.—The cells of the kidney showed various degrees of degeneration, ranging from parenchymatous to fatty infiltration. There were a few capillary hæmorrhages and hyaline casts present.

Suprarenals.—These showed slight degeneration.

Spleen.—This organ showed congestion, a few hæmorrhages, and bacterial emboli; otherwise normal.

The bacteriological examination of the material from this cat gave the following results:

1. *Smears:*
   - *a.* From the buboes showed degenerated leucocytes, many lymphocytes, and numerous bacteria, some of which resembled *Bacillus pestis* in their polar staining.
   - *b.* From the spleen showed numerous plague-like, polar-stained bacilli. Round involution forms were present.
2. *Cultures:*
   - *a.* From the buboes were badly contaminated with *Bacillus coli* and *Bacillus pyocyaneus* colonies.
   - *b.* From the spleen: A few scattered colonies of *Bacillus pyocyaneus* developed on the surface of the agar. Between the large colonies a scanty growth of dewy appearance was noticed. Smears made from this growth revealed plague-like bacilli of the cultural type, showing a few club-shaped involution forms. Subcultures were made in order to secure pure culture. They showed a pure growth of *Bacillus pestis* as indicated by the morphology of bacilli and shape of the colonies. Agglutination with plague-immune serum was positive.

3. *Inoculation experiments (vaccination method)*:-

a. One guinea pig was inoculated with the material from the left bubo, another one with material from the right bubo. They died of plague on the third and fifth days, respectively.

b. One guinea pig was inoculated with the material from the spleen. It died of plague on the third day.

c. One guinea pig was inoculated with material from the nostrils obtained by swab. The animal survived, showing no indication of plague.

d. One guinea pig was inoculated with material from the rectum obtained by swab. It died of plague on the fifth day.

Although plague infection among cats is apparently a rare occurrence, the fact that cats may contract the disease in spite of the high degree of resistance to plague infection has to be considered from the hygienic standpoint.

To appreciate the important rôle which cats may play in the spreading of the disease one need only consider the close contact of these animals with rats on one side and human beings on the other. It is also a well-established fact that not only plague-infected cats, but also those which have devoured plague-infected material and remained apparently normal, may excrete plague bacilli which have retained their full virulence.

ILLUSTRATION

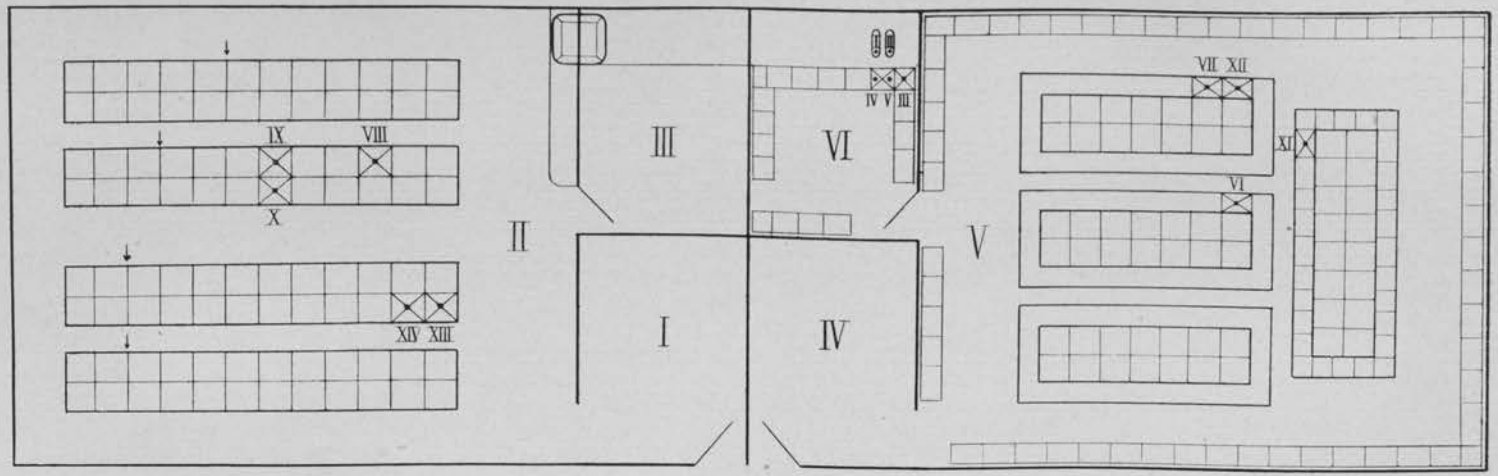

PLATE I. ANIMAL HOUSE.

SOME EXPERIMENTS ON THE INOCULATION OF MONKEYS WITH SMALLPOX

By P. M. ASHBURN, E. B. VEDDER, and E. R. GENTRY [1]

(The United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

Seven charts

I. EXPERIMENTS WITH VESICLE CONTENTS FROM A CASE OF DISCRETE SMALLPOX

On December 4 the case of a Dutch traveler who had contracted smallpox in China came under observation. This case was a very typical discrete smallpox in a man whose general condition was excellent and who had been successfully vaccinated in childhood (about 1884) and revaccinated with doubtful result about 1900. At the time of admission he was moderately covered with discrete lesions, those on the upper part of the body being good vesicles, those on the feet and legs not quite mature. He was in the eighth day of the disease. Vesicle contents drawn into capillary tubes was used to inoculate 5 monkeys. Other vesicle contents in capillary tubes was preserved for later use.

Monkey 21.—This animal, an unvaccinated female, was inoculated on the belly on December 5. The temperature (101°.4 F. at time of inoculation) rose steadily until the 11th when it reached 104°.8 F., dropping by the morning of December 12 to 95°.4 F. The animal died on the 12th, the cause of death being sepsis from a large abscess below the jaw, which was doubtless due to an injury received in fighting with other monkeys before the inoculation. The sites of inoculation on the belly showed waxy scabs, not to be definitely described as "takes."

Monkey 5.—A large male monkey, that had been successfully vaccinated in October, was inoculated at 6 sites on the abdomen on December 4 with fresh vesicle contents. No local lesions resulted. There was, however, a moderate rise of temperature

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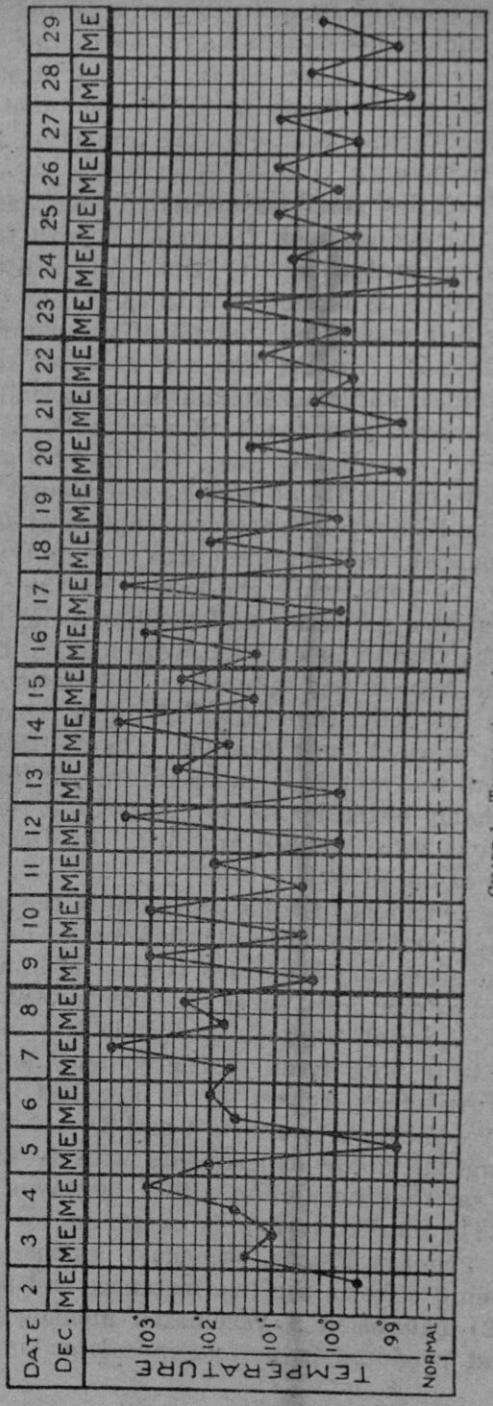

CHART 1.—Temperature chart of monkey 5.

on the third day, followed by a drop, and a second rise on the sixth day, with almost continuous elevation to the seventeenth day. The chart (chart 1) is attached. We call attention to the probability of this rise being due to *variola sine eruptione,* the eruption being absent because of the protection afforded by the vaccination in October.

Monkey 19.—A rather small unvaccinated male was inoculated at 5 points on the abdomen on December 4 with fresh vesicle contents. The temperature chart (chart 2) is attached, and attention is invited to its resemblance to chart 1.

On December 8 it was noted that all of the points of scarification were reddened. On the 10th the areas of redness were more widely extended and marked, and the belly wall about them was deeply indurated. On the 11th dry scabs were forming and the induration was slightly less. On the 12th a papule was noted on the scalp; on the 13th 5 papules were found on the scalp and legs. By the 15th a fairly profuse eruption of small vesicles and pustules, some of them ruptured, was seen on the face, arms, legs, and about the anus, while the lesions on the abdomen had further subsided, the induration and swelling about them greatly lessened, and the sites of inoculation were marked by dry scabs. On the 17th the redness and induration had almost entirely disappeared from the belly, the scabs had fallen from the inserts, and deep holes marked their location. On the 19th the end of the monkey's tail was seen to be much injured, as though crushed or bitten (probably bitten by an old male, No. 5, tied near), and all variola lesions were scabbed and dry. By December 23 the animal was pronounced well.

This case we considered *variola inoculata* in the monkey, characterized by fever and signs of local inflammation on the fifth day, by primary and secondary eruptions (the latter appearing on the ninth day), and by continued fever for about seventeen days.

Monkey 20.—A small unvaccinated male was inoculated at 4 points on the belly with fresh vesicle contents on December 4. The temperature chart (chart 3) is attached, and attention invited to its resemblance to charts 1 and 2.

Summarizing the above experiments with fresh vesicle contents, we may say that inoculation with it, by way of scarifications of the skin: (1) caused *variola inoculata* in two unvaccinated monkeys, the primary lesions, secondary lesions, and temperature curves being alike in the two instances and probably characteristic; (2) caused in a vaccinated monkey a fever very similar to that produced in *variola inoculata,* but gave rise to

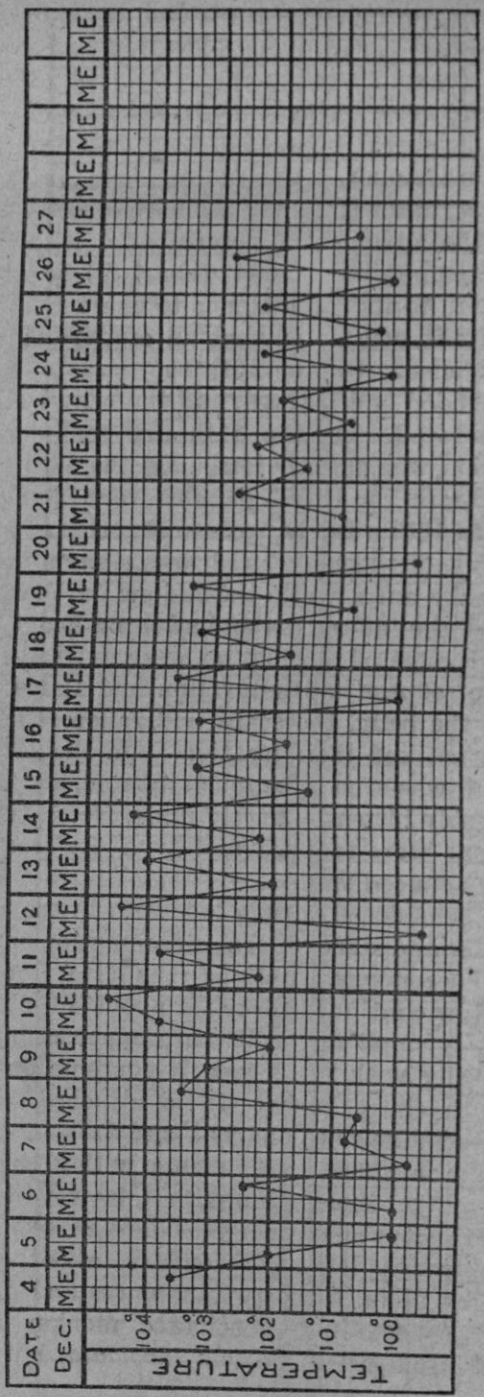

CHART 2.—Temperature chart of monkey 19.

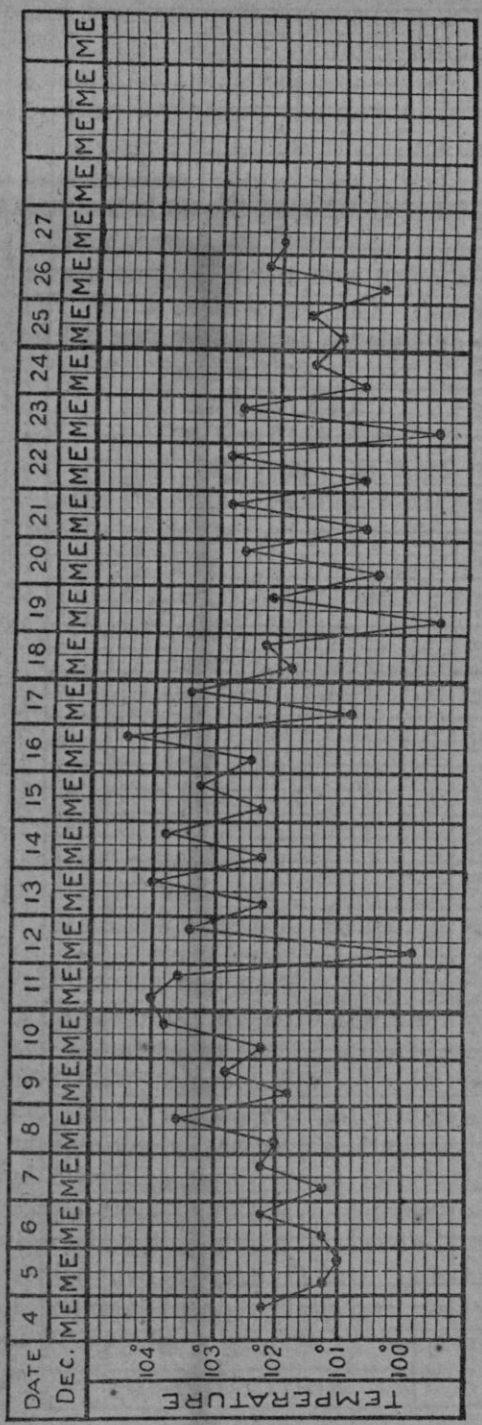

CHART 3.—Temperature chart of monkey 20.

neither primary nor secondary skin lesions. This fever might well be the manifestation of *variola sine eruptione*. Placed free on the mucous membranes of the conjunctivæ, nares, and mouth, the virus caused no disturbance, or, if any, so little as to be insufficient for interpretation as an evidence of infection.

Of the fresh vesicle contents tubed and not used on the above monkeys, the greater part, probably 20 tubes, was used for the inoculation, by scarifications and intravenously, of 2 horses. Neither animal showed symptoms or signs that could be interpreted as smallpox. The remainder, which was partly clear vesicle contents and partly contents drawn on December 7 and

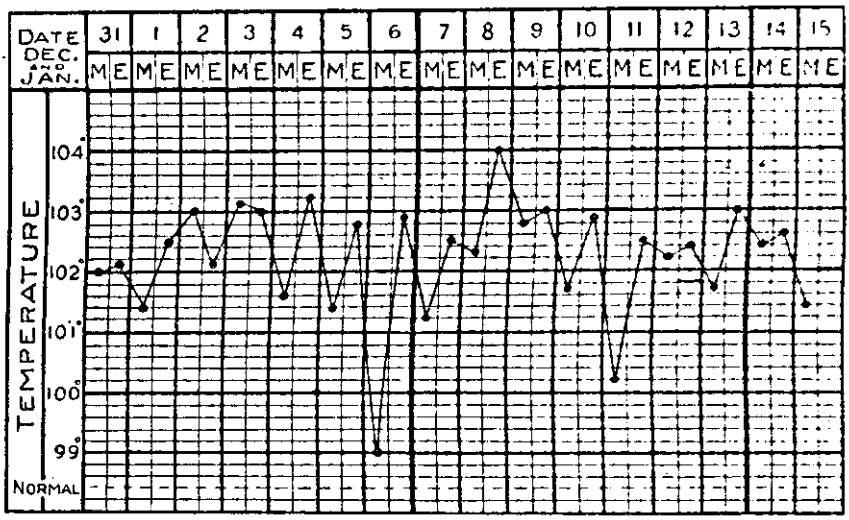

CHART 4.—Temperature chart of monkey 30.

showing slight turbidity, was kept in an ordinary ice chest for twenty-four days and was then used to inoculate 2 monkeys.

Monkey 30.—A medium-sized unvaccinated male was inoculated at several sites on the abdomen with 24-day-old vesicle contents on December 31. On January 7 an enlargement of a right inguinal lymphatic gland was noted, and on the 8th there was a transient rise of temperature as shown by chart 4.

Monkey 23.—A large unvaccinated male monkey was inoculated December 31 at several points on the abdomen with 24-day-old vesicle contents. On January 6 five points and lines of induration, swelling, and slight redness were noted about inserts, and the temperature was elevated as shown by chart 5.

By the 8th the induration, redness, and swelling were all beginning to diminish. Dry scabs covered the points of insertion. No secondary lesions developed. On January 7 some of these scabs were raised and the beds on which they rested scraped. These scrapings and the triturated scabs were used to inoculate monkeys 8 and 16.

Summarizing the above we may say that vesicle contents, capable when fresh of causing *variola inoculata* in monkeys, so loses its virulence by being kept for twenty-four days in the

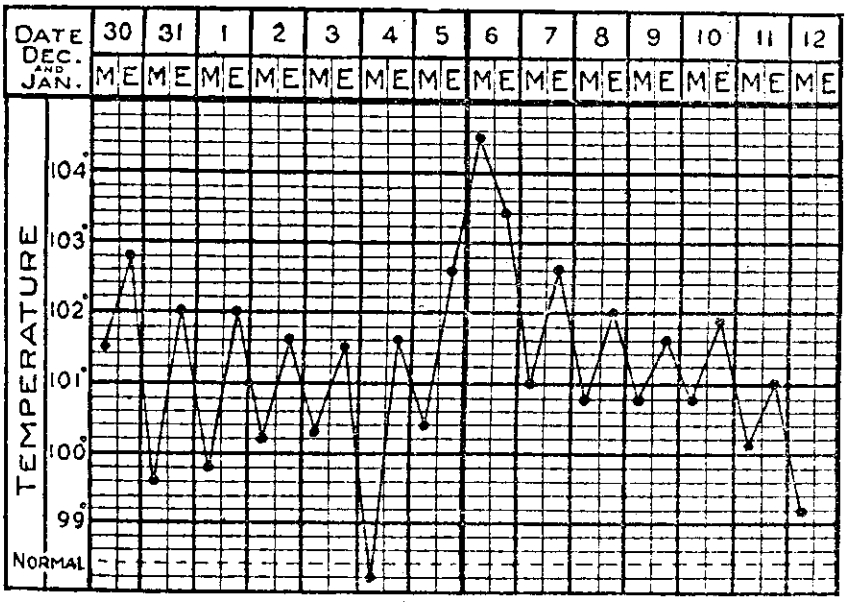

CHART 5.—Temperature chart of monkey 23.

ice chest as to be no longer capable of producing the typical disease with prolonged fever and primary and secondary lesions.

It did produce an ephemeral rise in temperature in both instances after an incubation period prolonged beyond the ordinary length, and in one of the two instances it gave rise to abortive and atypical primary lesions. In neither instance did secondary lesions or severe disturbance result.

The above finding would indicate that a working and satisfactory smallpox prophylactic might be secured by storage and attenuation of virulent vesicle contents, but prophylaxis by vaccination as practiced is so safe, satisfactory, and efficient that the pursuit of the clue appears at present unnecessary.

II. EXPERIMENTS WITH SCABS OR "DISKS" FROM THE ABOVE CASE OF SMALLPOX IN MAN

As the lesions on the person of the Dutch traveler matured and the scabs fell or were picked off, they were all collected and saved; one-half of them were placed in glycerin and one-half were placed dry in a sterile test tube.

On December 19, the patient's sixteenth day in the hospital and about the twenty-third day of his sickness, some of each lot of scabs were triturated in saline solution and some with the serum of monkey 6 (a vaccinated monkey), so as to make thick suspensions. With these suspensions monkeys 28, 22, 26, 27, and 29 were inoculated, 5 or 6 insertions being made on the belly of each.

Monkey 28.—This monkey received scabs preserved in glycerin and triturated with vaccinated monkey's serum. No local lesions developed. On the eighth and tenth days the monkey showed sharp rises of temperature, as indicated by chart 6. He thereafter appeared well.

The sites of inoculation were first reddened on December 8; on the 10th the redness and induration were very marked, as in monkey 19. On the 11th small vesicles or pustules marked the insertions, and two of them were ruptured. The next day the swelling and redness had begun to subside and the lesions were scabbed. On the 13th small secondary lesions, papules, were seen on the legs and about the anus. On December 15 a profuse eruption of small vesicles and pustules, more numerous than in monkey 19, was present on the palms, arms, legs, face, and scalp. The abdominal lesions were subsiding, and the inflammatory process in the abdominal wall was almost gone. On the 17th the belly wall was more inflamed and indurated and the swollen ridges were black on top; apparently secondary infection had occurred. All the secondary lesions were either pustules or scabs. On December 18 the tops of the swollen ridges on the belly sloughed, leaving extensive ulcers, and it may here be stated that these ulcers were not completely healed until the end of the month. Numerous pustules of the secondary lesions were yet unscabbed, but by December 21 all had become so, and desquamation was completed by the 26th, the completion being delayed on the palms, where the disks were held down by thickened epidermis, and on the legs, where entanglement of hairs in the scabs doubtless delayed it.

This case we also regard as one of *variola inoculata* in the monkey, characterized by fever and signs of local inflammation

on the fifth day; by primary and secondary eruptions, the latter appearing on, or escaping notice until, the tenth day; and continued fever until the fifteenth and possibly the nineteenth day.

Monkey 12.—A medium-sized unvaccinated monkey was given

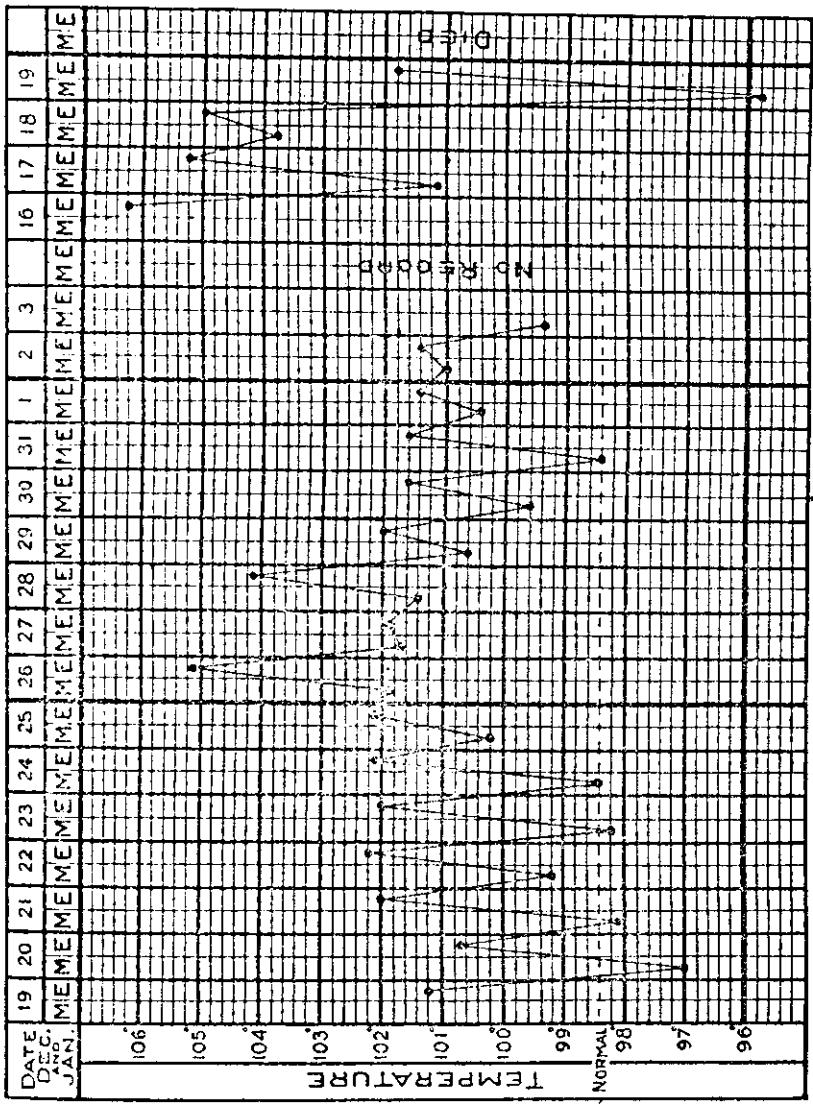

CHART 6.—Temperature chart of monkey 28.

a drop of fresh vesicle contents in each eye, each nostril, and each side of the mouth on the morning of December 5. The virus was placed free on the mucous surfaces. No local lesions resulted, and no systemic disturbance other than a trifling rise

of temperature on the sixth, seventh, and eighth days, and we are unable to affirm that any infection occurred.

On January 16 he was again found to be sick and to have a high temperature, and on January 19 he died. Autopsy showed streptococcus septicæmia as the cause of death. That the sharp rises in temperature on December 26 and 28 were related to the septicæmia that caused death three weeks later, notwithstanding the interval of apparent health and normal temperature, is possible.

Monkey 22.—Inoculated with dry scabs triturated in 0.85 per cent saline solution; this monkey showed no reaction, either local or general.

Monkey 26.—Inoculated with glycerinated scabs; this monkey showed no reaction.

Monkey 27.—Inoculated with glycerinated scabs; showed no reaction.

Monkey 29.—Inoculated with glycerinated scabs; showed no reaction.

On December 24 monkeys 12 and 17 were inoculated at 6 points on the belly with dried scabs, and monkey 33 with both dry and glycerinated scabs. None of them showed general or local disturbance.

III. EXPERIMENTS WITH SCABS FROM A CASE OF RECOVERED VARIOLOID ON THE SIXTEENTH DAY

In addition to the case of smallpox above mentioned, the United States Army transport *Sherman* arrived in port on December 1, 1912, with a naval recruit in his sixteenth day of modified smallpox, which he had contracted in San Francisco and developed after leaving Honolulu. The attack had been mild, the lesions abortive, and at the time of his arrival here the man showed only a few small, dry, brown scabs. These were all collected, and on December 2 were triturated in sterile 0.8 per cent salt solution and used to inoculate 3 monkeys, Nos. 6, 17, and 18.

Monkey 6.—This animal had been successfully vaccinated in October. No lesions followed inoculation with the scabs. The animal had an irregular temperature from the first and was sickly. On December 18 it was killed, in order to get vaccine immune serum.

Monkey 17.—A medium-sized female, unvaccinated, showed no disturbance and no lesions as a result of the inoculation.

Monkey 18.—A small unvaccinated male showed neither lesions nor systemic disturbance as a result of the inoculation. He was later (December 24) successfully vaccinated.

IV. EXPERIMENTS WITH SCABS FROM VARIOLOUS MONKEYS

While monkeys 19 and 20 were suffering from their variola, attempts were made to obtain vesicle contents from them, but the vesicles were so small and so soon ruptured by the animals that it was found impracticable. Scabs were collected, however, as the lesions dried, and these were used to inoculate monkeys 24, 25, 30, and 31. The results in all of these animals were quite negative, with the exception of monkey 24.

Monkey 24.—A medium-sized female was inoculated December 19 with scabs from monkeys 19 and 20. No general or febrile disturbance resulted, but on December 26 there was swelling, redness, and marked induration of 3 points of insertion and their surroundings. The lesions formed dry scabs. The induration persisted about ten days, and the monkey remained well.

Whether or not the above monkey suffered from modified primary lesions of smallpox we cannot know positively, but it seems probable. At any rate, all of the above experiments with smallpox scabs or disks from man and monkeys indicate that such material has but feeble virulence and that such as it has is speedily lost.

On January 7, 1913, the scabs were lifted from the lesions on monkey 23 (see above), the underlying tissue curetted, and the pulp so obtained used to inoculate monkeys 8 and 16.

Monkey 8.—A monkey that had been vaccinated in October with the vaccine scab from a pig, atypical but supposedly successful "takes" having been obtained, showed redness and slight swelling at the points of inoculation with pulp from No. 23, but nothing at all characteristic or strongly suggestive of smallpox or vaccinia.

Monkey 16.—This monkey had been unsuccessfully inoculated in November with vaccine triturated in 1 per cent phenol in 0.85 per cent saline solution and so kept for two weeks, no "take" resulting. Inoculated with "pulp" from the lesions of monkey 23 on January 7, the animal had a rise of temperature beginning the sixth day thereafter as shown by chart 7.

Beginning on the seventh day after inoculation, the animal showed marked induration and some œdema of and about the sites of inoculation and thick dry scabs formed. The induration was deep. On January 16 (tenth day after inoculation) the scabs were lifted, the areas beneath curetted, and the pulp so obtained used to inoculate monkeys 25, 19, 28, and 3, the first three of which have been discussed, and the last being a monkey vaccinated in October. None of them showed general or local disturbance.

This experiment indicates that the virus in vesicle contents, although attenuated by storage and further attenuated by passage, was still recognizably active in this animal, but not sufficiently so to survive another passage.

SUMMARY

1. Fresh vesicle contents from a case of human variola is capable, when inoculated into abrasions or scarifications on non-vaccinated monkeys, of producing *variola inoculata* in those monkeys, the disease being marked by fever and by primary and secondary lesions.

2. Such vesicle contents kept at ice-chest temperature for twenty-three days loses most of its virulence, but may still, in

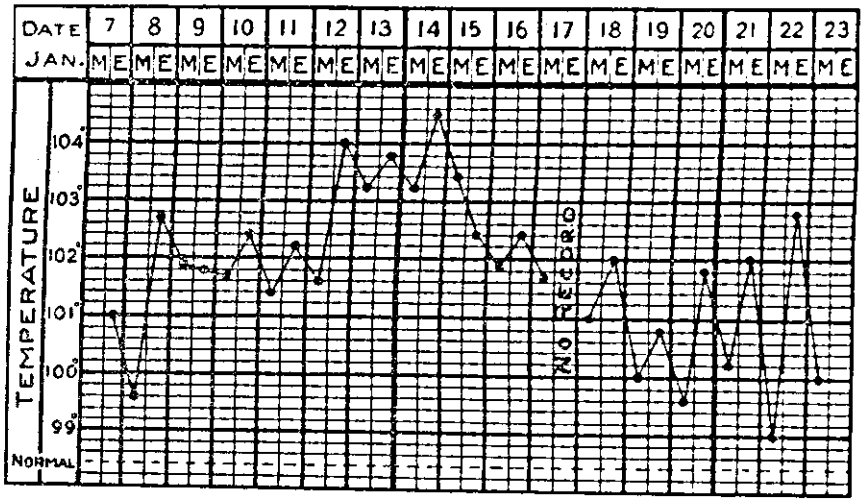

CHART 7.—Temperature chart of monkey 16.

a proportion of instances, produce a mild and atypical *variola inoculata*, which in turn and in further modified form may be passed to other monkeys.

3. Active and fresh vesicle contents inoculated on vaccinated monkeys may produce a fever closely resembling that of *variola inoculata* in the monkey and a condition permitting of interpretation as *variola sine exanthemate* in the monkey.

4. Smallpox scabs or disks from man or monkey possess but a low degree of virulence, or very quickly lose their virulence.

5. When inoculation of such scabs does result in the production of infection this may be manifested only locally at the site of inoculation (case 24). In other words, the "B" part of smallpox virus survives longest in scabs.

COMMENT

We admit that this small series of experiments affords but little proof of the correctness of our hypothesis as to the relationship of variola and vaccinia.[2] On the contrary, we do not see that it affords any evidence in disproof. The case of monkey 5, although of little value standing alone, is certainly susceptible of being cited as an instance of *variola sine exanthemate*, as an instance of separation of the elements of smallpox virus (the pock-producing or "B" part having acted on the monkey in October; the toxæmia-producing, pyrogenic, or "A" element in December), and as proof that vaccination protects against the pock-forming element of smallpox rather, or to a greater degree, than against the whole disease. We feel justified in restating our hypothesis that smallpox is due to a dual and divisible virus, one part of which is the cause of vaccinia and the pock stage of smallpox, the other part being necessary for the production of the highly contagious, febrile, general disease with an initial stage and preliminary rashes.

[2] *This Journal, Sec. B* (1913), 8, 17–28.

A BACTERIOLOGICAL EXAMINATION OF CERTAIN ARTESIAN WELLS IN RIZAL, CAVITE, AND BULACAN PROVINCES, P. I.

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Since artesian wells have become one of the chief sources of potable water in Rizal, Cavite, and Bulacan Provinces, P. I., it was thought advisable to make a careful bacteriological test of a number of them for the presence of pollution. In addition, it seemed of scientific interest to compare the numbers of bacteria found in different waters from these sources. These wells may fairly represent similar wells in these and other provinces. In all, 34 wells were examined, 22 of them flowing and 12 pumping. Some of the pumping wells were formerly flowing, and in all flowing wells, as a rule, there has been a diminution in the rate of flow since the wells were sunk. Among the 12 pumping wells are included 2—Malolos (Plaza Malolos) and Bocaue—which flow intermittently at the present time. In some of the wells the flow is said to be greater at high tide.

In practically every case nutrient media were taken to the well itself, and the water transferred to most of the tubes or plates directly from the source. Whenever practicable, the tip of the sterile pipette was brought directly into the flowing stream in taking samples. Usually an additional sample was packed in ice and transported to the laboratory for additional or confirmatory tests. It was early found that the water of the flowing wells was nearly sterile; so, in order to get some comparisons, a larger range of tests was made and larger quantities of water sown than an ordinary sanitary analysis would require.

In all agar tests the water was well mixed with liquified agar cooled to 42° C. Plate cultures were made only where stated in the table. Plates were kept at high room temperature (28° to 32° C.). All other cultures were incubated three days, then removed to room temperature. The rate of flow was estimated by noting the time required to fill a can of approximately 5 gallons' (19 liters') capacity.

SERIES I. FLOWING WELLS

The results obtained from the flowing wells were so uniform that it is not necessary to tabulate the results of all the 22 in the series. In order to give an idea of the method employed and the character of the results, the findings in two of them, the one showing the highest degree of purity and the one showing the lowest, are given in full. One well gave a slightly lower degree of purity than the one chosen, but that well gave brackish water and was little used for drinking; so the next to the poorest is tabulated. The well showing the highest degree of purity was at Malolos, barrio Caingin. Rate of flow, 49 liters per minute; temperature of water, 28°.4 C.; standing water near well from overflow; no houses near.

TABLE I.—*Water from well in the barrio of Caingin, Malolos, Bulacan Province.*

Amount of water sown.	Nutrient medium.	Result.[a]											
		20 hours at 36°.			40 hours at 36°.			3 days at 36°.			6 days, 3 at room temperature.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
1	Glucose-broth fermentation tube	0	----	0	0	----	0	0	----	0	0	----	0
5	----do	0	----	0	0	----	0	0	----	0	0	----	0
1	Glucose agar	0	----	0	0	----	0	0	----	0	0	----	0
5	----do	0	----	0	0	----	0	0	----	0	0	----	0
1	Plain agar	0	----	0	0	----	0	0	----	0	0	----	0
5	----do	0	----	0	0	----	0	0	----	0	0	----	(b)
5	Litmus-lactose agar	0	0	0	0	0	0	0	0	0	0	0	0
5	Bile-lactose agar	0	----	0	0	----	0	0	----	0	0	----	0
1	Plain agar-plate culture	----	----	----	----	----	(c)	----	----	----	----	----	----

[a] Amœba test negative. [b] Growth on surface only. [c] One colony near margin.

The well opposite the municipal building at Meycauayan gave the lowest degree of purity. This water was slightly brackish, and was reputed to have medicinal virtues. It is generally used for drinking purposes. Rate of flow, about 5.7 liters per minute; temperature, 29° C.

TABLE II.—*Water from well opposite the municipal building, Meycauayan, Bulacan Province.*

Amount of water sown.	Nutrient medium.	Result.[a]											
		20 hours at 36°.			40 hours at 36°.			3 days at 36°.			6 days, 3 at room temperature.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
1	Glucose-broth fermentation tube	0	.....	0	0	.....	0	0	.....	0	0	.....	0
5	....do	0	.....	.....	0	.....	.....	0	.....	.....	0	.....	.....
1	Glucose agar	0	.....	(?)	0	.....	.....	0	.....	.....	0	.....	(b)
5	....do	0	.....	(?)	0	.....	.....	0	.....	.....	0	.....	(c)
1	Plain agar	0	.....	0	0	.....	0	0	.....	0	0	.....	0
5	....do	0	.....	0	0	.....	.....	0	.....	.....	0	.....	(d)
5	Litmus-lactose agar	0	0	0	0	0	0	0	0	0	0	0	0
5	Bile-lactose agar	0	.....	0	0	.....	0	0	.....	0	0	.....	1
1	Plain agar plate	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....	.....
5	Broth 1 cc	Apparently a pure culture of a zoöglœa-forming bacterium.											

[a] Amœba test negative.
[b] +3 or more.
[c] +4 or more.
[d] 3 or more.

In Table III is given a list of all the flowing wells examined with a part of the data obtained from each. A complete examination was made of all of these, and some of them were examined twice; but, as stated above, the results were so uniform that it is not necessary to give them in detail. Under number of colonies per cubic centimeter the entries "one or less," "two or less," etc. mean that the maximum number found in any one cubic centimeter sample was one or two, respectively. Where "one or more" is entered, some very small number above one was found in some tube where an exact determination was difficult. In every water there was at least one of the 1 cubic centimeter samples which remained sterile after five days or more, and in most cases several samples of 5 cubic centimeters remained sterile.

Results such as are exhibited by Table I raise the questions as to whether or not the water as it comes from the source is absolutely sterile and whether or not the few growths which appeared in the media are contaminations. In practically every well some algæ were growing inside the mouth of the exit tube and under the stream of water, and it may be from colonies of bacteria among them that some of the growths came.

In a well (Malolos, barrio Mambong) flowing 23 liters per minute, samples were taken, and afterward the mouth of the exit tube was thoroughly scrubbed with stiff test-tube brushes. Four and one-half hours later a second lot of samples was taken. Both lots of samples gave a high degree of purity, but the second was not superior to the first. This experiment was repeated with another well (Paombong, church square) having a flow of 57 liters per minute. Here only a few minutes intervened between the first and second tests. There was little difference between the two tests; both were nearly, but not quite, sterile. Four cubic centimeters of ordinary broth were added to 10 cubic centimeters of this water and the mixture remained clear two days at high room temperature, becoming clouded only on the third day.

Even if all sources of contamination within reach could be eliminated, there would still be a possibility of contaminants growing on the casing deep in the ground. Further, before absolute sterility could be proved, it would be necessary to employ all sorts of media, nonnitrogenous and otherwise, in order to eliminate every possible kind of bacterium; and there would still remain the possibility of the presence of thermophiles of different grades and of forms unable to grow on any artificial medium. It would, therefore, be a difficult, if not impossible, task to prove absolutely the sterility of a water of this sort. The relative freedom of these waters from bacteria seems the more remarkable when we consider that they are at a temperature (28° to 32°) which is very favorable to the growth of most bacteria.

A number of kinds of bacteria were found in these wells, but the commonest type was an actively motile bacillus, readily forming zoöglœa.

2. PUMPING WELLS

While the flowing wells examined are located near the coast, most of the pumping wells are 8 kilometers or more inland; although two of them are near the coast and in the immediate neighborhood of flowing wells.

The results of these tests are given in detail in Tables IV to XVI, inclusive. Samples were for the most part placed in the media at the well, but some samples were taken to the laboratory for further examination, as was done in the case of the flowing wells. Enough water was pumped out to flush out the pipe before samples were taken, although the wells were in continuous use during the time of day when samples were collected. Two wells which flowed intermittently are included in this list.

In Tables IV to XVI the fractions under the heading "gas" refer to the proportion of gas formed in the closed arm of the fermentation tube.

TABLE IV.—*Alabang Agricultural Experiment Station, Rizal Province, Well 1.*

Water, fresh; depth of wells, 167 meters; in use since July, 1908. Water of several wells pumped to collecting tank. Samples (taken April 11, 1913) from pipe leading from collecting tank.

Amount of water sown.	Nutrient medium.	Result.											
		24 hours at 36°.			48 hours at from 36–37°.			3 days at from 36–37°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
1	Glucose-broth fermentation tube	0		+	0		+	0		+	0		
5	do	0		+	0		+	0		+	0		+
1	Glucose agar	0		+	0		+	0		(a)	0		(b)
5	do	0		(c)	0		(c)	0		(c)	0		+
1	Plain agar	0		+	0		(d)	0		(d)	0		+5
1	do	0		+	0		(e)	0		(e)	0		(f)
5	do	0		(c)	+		+			+	+		+
1	Litmus-lactose fermentation tube	0	0	+	0	0	+	0		+	0	+	+
5	do	+	0	+	2/10	0	+	2/10		+	2/10	+	+
1	Litmus-lactose agar	0	0	+	0	0	+	0	0	+	0	0	+
5	do	0	0	+	0	0	+	0	0	+	0	0	+
1	Bile-lactose fermentation tube	0		0	0		0	0		0		0	0
5	do	0		+	0		+	0		+	0		+
1	Litmus-lactose agar plate									1			

[a] + about 3.
[b] + 5 or more.
[c] Many colonies present.
[d] + about 4.
[e] + about 6.
[f] + about 7.

TABLE V.—*Alabang Experiment Station, Rizal Province. Well 2.*

Samples taken (April 11, 1913) from pipe leading directly from well—not through collecting tank; water, sweet; depth, 231 meters; in use since October, 1908.

Amount of water sown.	Nutrient medium.	Result.											
		24 hours at 36°.			48 hours at from 36–37°.			3 days at from 36–37°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
0.1	Glucose-broth fermentation	0	.....	+	0	.....	+	0	.....	+	0	.....	+
1	do	1/3	.....	+	1/3	.....	+	1/3	.....	+	1/3	.....	+
5	do	1/5	.....	+	1/5	.....	+	1/5	.....	+	1/5	.....	+
0.1	Glucose agar	0	.....	+	0	.....	(a)	0	.....	+	0	.....	+
1	do	0	.....	+	0	.....	(b)	0	.....	+	+	.....	+
5	do	+	.....	+	+	.....	+	+	.....	+	+	.....	+
1	Plain agar	0	.....	+	0	.....	(c) 120/230	0	.....	+	0	.....	+
0.1	Litmus-lactose agar	0	0	+	0	0	+	0	0	+	0	0	+
1	do	0	0	+	0	0	+	0	0	+	0	0	+
5	do	0	0	+	(d)	0	+	+	0	+	+	0	+
0.1	Litmus-lactose broth fermentation tube	0	0	+	0	0	+	0	0	+	0	0	+
1	do	0	0	+	1/10	0	+	1/10	+	+	1/10	(e)	+
5	do	3/10	0	+	5/10	0	+	5/10	0	.....	5/10	0	+
0.1	Bile-lactose-litmus fermentation tube	0	.....	+	0	.....	+	0	.....	+	0	.....	+
1	do	0	.....	+	0	.....	+	0	.....	+	(?)	.....	+
5	do	0	.....	+	0	.....	+	0	.....	+	(?)	.....	+
1	Plain agar plate[f]	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] 6 or more.
[b] Many colonies present.
[c] From 100 to 200 colonies.
[d] Slight.
[e] Neutral.
[f] Three days at room temperature, 236 colonies.

TABLE VI.—*First examination of water from well in the church square, Bocaue, Bulacan Province.*

First examination, March 8, 1913. Now flowing 3.8 liters per minute; said to flow most at high tide; water, sweet; temperature, 29° C.; ditch with standing water near.

Amount of water sown.	Nutrient medium.	Result.[a]											
		20 hours at 36°.			40 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
1	Glucose-broth fermentation	5/10		+	5/10		+	7/10		+	7/10		+
5	do	9/10		+	10/10		+	10/10		+	9/10		+
1	Glucose agar	0		0	0		1	0		1	0		1
5	do	+		(b)	+		(b)	+		(b)	+		(b)
1	Plain agar	0		0	0		0	0		0	0		0
5	do	0		(b)	+		+	+		+	+		+
5	Litmus-lactose agar	+	(?)	(b)	+	+	+	+	+	+	+	+	+
5	Bile-lactose agar	+		(b)	+		+	+		+	+		+

[a] Amœba test negative. [b] Many colonies present.

TABLE VII.—*Second examination of water from well in the church square, Bocaue, Bulacan Province.*

Second examination, March 27, 1913. Water now being raised by a pump.

Amount of water sown.	Nutrient medium.	Result.[a]								
		48 hours at 36°.			65 hours at 36°.			7 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation tube	1/10		+	5/10		+	7/10		+
1	do	5/10		+	5/10		+	5/10		+
5	do	7/10		+	7/10		+	9/10		+
0.1	Glucose agar	0		1	0		1	0		1
1	do	+		+	+		+	+		+
5	do	+		+	+		+	+		+
1	Plain agar	+		+	+		+	+		+
5	do	+		+	+		+	+		+
5	Litmus-lactose agar	+	0	+	+	+	+	+		+
1	Bile-lactose agar	0		+	0		+	+		+
5	do	+		+	+		+	+		+
1	Plain agar-plate culture			15						

[a] Amœba test negative.

This water is in all probability polluted. From the 0.1 cc. sample in the glucose-broth fermentation tube a bacillus was isolated with coli-like morphology, motility doubtful. It produced acid and gas in lactose-, dulcite-, saccharose-, and mannite-litmus agars, and gas in bile-lactose agar. Nine-tenths of the closed arm of a glucose-broth fermentation tube was filled with gas, about half of which was absorbed by NaOH. See Table XVII.

TABLE VIII.—*Water from wells at Fort William McKinley, Rizal Province.*[a]

Samples taken April 9, 1913; water, sweet; temperature, 30° C.; near Pasig River; depth, from 225 to 274 meters; water collected from several wells, about 2,270,000 liters used daily; samples were taken from the main leading from the collecting tank to the post.

Amount of water sown.	Nutrient medium.	Result.											
		24 hours at 36°.			48 hours at 36°.			3 days at 36°.			8 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
0.1	Glucose-broth fermentation tube	1/10		+	1/10		+	1/10		+	1/10		+
1	do	1/10		+	1/10		+	1/8		+	1/8		+
5	do	1/10		+	1/10		+	1/10		+	1/8		+
1	Glucose agar	+		+	+		+	+		+	+		+
5	do	+		+	+		+	+		+	+		+
0.1	Plain agar	0		+	0		+	0		(b)	0		+
1	do	0		(c)	0		+	+		+	+		+
0.1	Litmus-lactose fermentation tube	+	+	+	1/10	(d)	+	1/10	-	-	1/10	+	+
1	do	+	(?)	+	1/10	(?)	-	1/10	(d)	-	1/10	(d)	+
5	do	+	(d)	+	+	(d)	+	1/10	(d)	+	1/10	(d)	+
5	Litmus-lactose agar	0	0	+	+	(d)	+	-	(d)	-	+	(d)	-
0.1	Bile-lactose fermentation tube	0		+	(e)		1	(e)		+	1/10		+
1	do	0		+	(e)		+	(e)		+	1/10		+
5	do	(e)		+	1/10		+	1/10		-	1/10		+
1	Litmus-lactose agar plate			(f)									
1	Plain agar plate			(g)									

[a] See Table XVII.
[b] + about 10.
[c] + about 100.
[d] Alkaline.
[e] Slight.
[f] 30+ (overgrown).
[g] 46+ (overgrown).

TABLE IX.—*Water from well in Plaza Malolos, Malolos, Bulacan Province.*

Sample taken March 18, 1913; water, sweet to taste; temperature, 29° C.; located on public street; not flowing spontaneously, but water could be siphoned from the pipe; depth, 64 meters; in use since 1908.

Amount of water sown.	Nutrient medium.	Result.[a]											
		20 hours at 36°.			40 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
1	Glucose-broth fermentation	0		+	0		+	0		+	0		+
4	do	0		+	0		+	0		+	0		+
1	Glucose agar	0		+	+		+	+		+	+		+
4.5	do	0		+	0		(b)	0		(b)	0		+
1	Plain agar	0		+	0		(b)	0		(b)	0		+
4	do	0		+	0		(b)	0		(b)	0		+
5	Litmus-lactose agar	0	0	+	0	0	(b)	0		(b)	0		+
5	Bile-lactose agar	0		+	0		(b)	0		(b)	0		+
1	Plain agar plate						38						

[a] Amœba test negative. [b] Many colonies present.

TABLE X.—*Water from well on Aglipay Street, Mariquina, Rizal Province.*

Samples taken March 28, 1913; water with slight mineral taste; temperature, 28° C.; very little standing water near; depth, 70 meters; in use three years.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation	0		0	0		0	0		0
1	do	2/10		+	2/10		+	2/10		+
5	do	0		+	0		+	0		+
1	Glucose-agar	0		0	0		0	0		+1
5	do	0		(b)	0		+	0		+
1	Plain agar	0		+3	0		+3	0		(c)
5	do	0		(b)	0		+	+		+
5	Litmus-lactose agar	0	0	(d)	0	0	+	0	0	+
1	Bile-lactose agar	0		0?	0		+1	0		+1
5	do	0		0	0		0	0		0
1	Plain agar plate			3						

[a] Amœba test negative. [b] Many colonies present. [c] +3 or 4. [d] +4 or more.

TABLE XI.—*Water from well near the municipal building, Mariquina, Rizal Province.*

Samples taken March 28, 1913; water with slight mineral taste; temperature, 28° C.; shallow ditch near; depth, 20 meters; in use since August, 1909.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation tube	1/10		+	1/10		+	1/10		+
1	do	1/10		+	1/10		+	1/10		+
5	do	1/10		+	1/10		+	1/10		+
1	Glucose agar	+		+	+		+	+		+
1	Plain agar	0		(b)	0		+	0		+
1	Litmus-lactose agar	+	+	(b)	+	+	+	+	+	+
5	do	+	+	+	+	+	+	+	+	+
1	Bile-litmus agar	0		(b)	0		+	0		+
5	do	0		+	+?		+	0		+
1	Plain agar plate			150						

[a] Amœba test negative. [b] Many colonies present.

TABLE XII.—*Water from well on Montalban Street, Montalban, Rizal Province.*

Samples taken March 28, 1913; water, sweet; temperature, 27° C.; standing water 2 meters away; depth, 19 meters; in use since May, 1909.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation tube	0		0	0		0	0		+
1	do	0		+	0		+	0		+
5	do	1/3		+	1/3		+	1/3		+
1	Glucose agar	0		(b)	0		+	0		+
5	do	+		(b)	+		+	+		+
1	Plain agar	0		(b)	0		+	0		+
5	do	0		(b)	0		+	0		+
5	Litmus-lactose agar	+	+	+	+	+	+	+	+	+
1	Bile-lactose agar	0		(b)	0		+	0		+
5	do	0		0?	0		0	0		(?)
1	Plain agar plate			3+						

[a] Amœba test negative. [b] Many colonies present.

TABLE XIII.—*Water from well near schoolhouse, Montalban, Rizal Province.*

Sample taken March 28, 1913; water, sweet to taste; temperature, 27° C.; standing water in a ditch about 6 meters away; depth, 28 meters; in use since May, 1909.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation	0		+	0		+	0		+
1	do	0		+	0		+	0		+
5	do	1/10		+	1/10		+	1/10		+
1	Glucose agar	0		(b)	0		+	0		+
5	do	+		+	+		+	+		+
5	do	0		+	+		+	+		+
1	Plain agar	0		+	0		+	0		+
5	do	0		+	0		+	0		+
5	Litmus-lactose agar	0	0	(b)	0	0	(b)	0	1	(b)
1	Bile-lactose agar	0		0	0		0	0		+1
5	do	0		(b)	0		+	+		+
1	Plain agar plate			1?						

[a] Amœba test negative. [b] Many colonies present.

TABLE XIV.—*Water from well on Arangu Street, San Mateo, Rizal Province.*

Samples taken March 18, 1913; water, brackish; temperature, 27°.7 C.; standing water from a drain at edge of well; depth, 46 meters; in use since April, 1909.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days at 36°.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation tube	0		0	0		0	0		0
1	do	0		+	0		+	0		+
5	do	1/10		+	1/10		+	1/10		+
1	Glucose agar	0		+	0		+	0		+
5	do	+		(b)	+		+	+		+
1	Plain agar	0		+3	0		(c)	0		(d)
5	do	0		(b)	0		+	0		+
5	Litmus-lactose agar	0	0	+	0	slight	+	0	0	0
1	Bile-lactose agar	0		+	0		+	0		+
5	do	0		+	0		+	0		+
1	Plain agar plate			1						

[a] Amœba test negative. [b] Many colonies present. [c] + 6 or more. [d] + 10 or more.

TABLE XV.—*Water from well in the church square, San Mateo, Rizal Province.*

Samples taken March 28, 1913; water has a slight mineral taste; temperature, 27°.8 C.; no standing water near; depth, 46 meters; in use since April, 1909.

Amount of water sown.	Nutrient medium.	Result.[a]								
		45 hours at 36°.			3 days at 36°.			6 days.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.										
0.1	Glucose-broth fermentation tube	1/10		+	1/10		+	1/10		+
1	do	1/10		+	1/10		+	1/10		+
5	do	1/10		+	1/10		+	1/10		+
1	Glucose agar	+		+	+		+	+		+
5	do	+		+	+		+	+		+
1	Plain agar	0		(b)	0		+	0		+
5	do	+		(b)	+		+	+		+
5	Litmus-lactose agar	+	−	+	+	+	+	+	+	+
1	Bile-lactose agar	0		(b)	0		+	+		+
5	do	+		(b)	+		+	+		+
1	Plain agar plate			(c)						

[a] Amœba test negative. [b] Many colonies present. [c] Overgrown.

TABLE XVI.—*Water from well in Taytay, Rizal Province.*[a]

Samples taken April 9, 1913; water, slightly brackish; temperature, 29°.5 C.; standing water only from overflow; depth, 158 meters; in use since June, 1911.

Amount of water sown.	Nutrient medium.	Result.											
		24 hours at 36°.			48 hours at 36°.			3 days at 36°.			8 days at 36°.		
		Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.	Gas.	Acid.	Colonies or cloudiness.
cc.													
0.1	Glucose-broth fermentation tube	0	----	+	0	----	+	0	----	+	0	----	+
1	----do	1/5	----	+	1/5	----	+	1/5	----	+	1/5	----	+
5	----do	1/3	----	+	1/3	----	+	1/3	----	+	1/5	----	+
1	Glucose agar	+	----	+	+	----	+	+	----	+	+	----	+
5	----do	+	----	+	+	----	+	+	----	+	+	----	+
1	Plain agar	0	----	+	0	----	+	0	----	+	0	----	+
5	----do	0	----	+	0	----	+	0	----	+	0	----	+
0.1	Lactose-broth fermentation tube	0	+	+	0	+	+	0	+	+	0	(b)	+
1	----do	0	+	+	0	+	+	0	+	+	0	+	+
5	----do	0	0	+	(c)	0	+	1/5	(b)	+	1/5	(b)	+
1	Lactose-bile fermentation tube	1/5	----	+	1/5	----	+	1/5	----	+	1/5	----	+
5	----do	0	----	+	0	----	+	(c)	----	+	1/5	----	+
5	Litmus-lactose agar	+	+	+	+	+	+	+	+	+	+	(b)	+
1	Litmus-lactose agar plate	----	----	[d]45	----	----	----	----	----	----	----	----	----
1	Plain agar plate	----	----	254	----	----	----	----	----	----	----	----	----

[a] See Table XVII. [b] Alkaline. [c] Slight. [d] 14 Acid.

Further tests were made of a number of samples which showed gas and acid in litmus-lactose agar or in bile media, in order to determine if *Bacterium coli* was present. Such samples from 6 different wells were transferred to litmus-lactose agar. No acid or gas appeared in stab cultures, although transfers were made directly from the original bile or litmus-lactose samples which had shown gas or gas and acid. In 3 wells colonies were isolated which showed gas and acid in litmus-lactose agar. These colonies were tested on various media, and the results are given in Table XVII.

TABLE XVII.—*Test of organisms producing gas and acid in litmose-lactose agar.*

Well.	Colony isolated from—	Morphology.	Motility.	One per cent glucose-broth fermentation tube gas.	One per cent bile-lactose fermentation tube gas.	Litmus agars containing 1 per cent of—											
						Lactose.		Saccharose.		Inulin.		Mannite.		Maltose.		Dulcite.	
						Gas.	Acid.	Gas.	Acid.	Gas.	Acid.	Gas.	Acid.	Gas.	Acid.	Gas.	Acid.
Bocaue:																	
First examination of well.	1 cc. sample in glucose-broth fermentation tube.	Colon-like	None after 4.5 hours glucose broth.	7/10	(a)	+	+	+	+	0	0	+	+	(b)	(c)	sl?	0
Second examination of well.	0.1 cc. sample in glucose-broth fermentation tube.	do	do	8/10	(a)	+	+	+	+	+	(?)	+	+	+	0	+	(a)
Taytay	1 cc. sample in bile-lactose fermentation tube.	do	do	1/8	(d)	+	+	+	+	0	0	+	+	0	0	(a)	0
Fort William McKinley:																	
(a)	1 cc. sample in litmus-lactose agar plate. Red colony.	Very fine bacillus not colon-like.	Active	1/8	0	(a)	(a)	+	+	0	0	+	(?)	(a)	0	0	0
(b)	0.1 cc. sample in litmus-lactose fermentation tube.	do	do	8/10	0	+	+	+	+	0	0	+	+	(a)	0 or (a)	0	0

[a] Slight, or 1/10 less. [b] Slight. [c] Slight or 0. [d] About 3/10.

Although some of these colonies resemble colon bacteria in some respects, it is evident that they vary somewhat from typical *Bacterium coli.*

From a comparison of the results obtained from the flowing and the pumping wells, it is evident that the pumping wells show a somewhat lower grade of bacterial purity. The best pumping well shows a greater degree of bacterial contamination than the poorest flowing well. It does not seem likely that the majority of the bacteria found in the pumping wells come from the deep water-bearing strata into which the wells are sunk, since flowing wells, in some cases only a few hundred yards away, show a much higher degree of purity; and it does not seem probable that there is seepage of surface water into these deep strata, since, in the two intermittently flowing wells, at least, there is pressure enough to bring the water near the surface. Neither is it probable that the source of contamination is in the pump. The Fort William McKinley pump, operated by machinery, raises about 600,000 gallons daily, and it is unlikely that such a volume of water would be much contaminated in passing through a pump with the ordinary protection from contamination. The waters of Alabang No. 1 and Alabang No. 2 are also raised by machinery, and the sample from No. 1 was taken from the pipe before it reached the collecting reservoir. These three wells showed about the same degree of contamination as those in which a hand pump was used.

All things considered, it seems probable that the source of contamination is water entering the wells above the deeper strata. In a flowing well, pressure is outward, and, obviously, there could be no inflow of water above the source, unless from strata in which the water is also under pressure. When the water in the well falls somewhat below the surface of the ground, as is the case in the pumping wells, the direction of the pressure in the upper part of the well, at least, is reversed and water may enter at any permeable point. The comparatively low degree of contamination in the pumping wells would indicate that such contaminating water enters in small quantities or is partially filtered before gaining access to the well.

It is improbable that any of the bacteria occurring in the pumping wells at the time of examination are dangerous to health. Fort William McKinley water is used unboiled by several thousand people, and there is no evidence that water-borne diseases come from its use. It is, of course, possible that some of these wells might become sources of disease under conditions other than those prevailing at the time of examination.

In summary, the waters from the flowing wells show a remarkable high degree of bacterial purity and may be regarded as safe from pollution by pathogenic bacteria. The pumping wells show a much lower degree of bacterial purity, although it is unlikely that any of them were polluted to a dangerous degree at the time of examination. These wells should be examined occasionally—especially during the prevalence of water-borne diseases—since they cannot be regarded as absolutely safe from pollution.

TABLE III.—*List of all the flowing wells examined.*

Town.	Province.	Location.	Date of taking sample.	Depth.	In use since—	Present rate of flow per minute.	Taste of water.	Temperature of water.	Surrounding of well.	Gas in—			Acid in litmus-lactose agar (5 cc. sample).	Colonies per cc.	Remarks.
										Glucose-broth fermentation (tube, 1 cc. sample).	Litmus-lactose agar (5 cc. samples).	Bile-lactose agar (5 cc. samples).			
			1913.	*Meters.*				°C.							
Caloocan	Rizal	Church square	Mar. 28	217	Sept., 1909	15 gallons	Slightly brackish	32.0	No houses near	0	0	0	0	1 or less	
Hagonoy	Bulacan	Plaza Burgos	Mar. 26	79	Oct., 1910	15 gallons	do (?)	29.5	No houses near. Estero 30 meter distant.	0	[a](?)	(?)	[a](?)	do	
Imus	Cavite	Church square	Mar. 14	98	July, 1910	Slow	Sweet		No houses near	0	0	0	0	2 or less	
Las Piñas	Rizal	do	Apr. 9	141	Apr., 1912	6 gallons	do	31.7	do	?	0	0	0	1 or less	
Malolos	Bulacan	Barrio Atlog	Mar. 18	33	Apr., 1908	5 gallons	Slightly brackish	28.5	About 6 meters from estero	+	0	0	0	5 or less	Gas in 1 cc. sample in glucose broth. Water said not to be used for drinking.
Do	do	Barrio Bagnua	Mar. 26	46	Apr., 1910	5 gallons	Sweet	28.5	No houses near	0	0	0	0	1 or less	
Do	do	Corner Burgos and Augustino Streets.	Mar. 18	[b]	1910	20 gallons	do	28.7	Almost under house	0	0	0	0	do	
Do	do	Barrio Cainĝin	do	61	Nov., 1910	13 gallons	do	28.4	No houses near	0	0	0	0	do	
Do	do	Barrio Mambong	do	46	do	6 gallons	do	28.3	About 12 meters from salt water	0	0	0	0	do	
Do	do	Barrio Santa Rosaria	do	63	do	5 gallons	do	28.5	Houses near	+	+	+	+	do	Gas and acid in litmus-lactose agar only after incubation for 3 days. Gas in bile agar after 6 days.
Marilao	do	Bath house	do	112	Jan., 1908	Very large flow	Brackish	30.0		0	0	0	0	3 or less	
Meycauayan	do	Opposite municipal building.	do	74	Nov., 1907	1.5 gallons	do	29.0		0	0	0	0	3 or more	
Do	do	No. 2	do	102	Nov., 1910	Very large flow	do	29.6		0	0	0	0	1 or less	
Novaleta	Cavite	Railway station	Mar. 14	160	Dec., 1909	Large stream			Houses within 12 meters	0	0	0	0	2 or less	
Paombong	Bulacan	Church square	Mar. 26	90	Dec., 1910	15 gallons	Brackish (?)	29.5	3 to 6 meters from estero	0	0	0	0	1 or less	
Do	do	Near schoolhouse	do	89	do	About 15 gallons	Brackish	29.2	Houses near	0	+	0	+	5 or less	
Parañaque	Rizal	Church square	Apr. 9	305	Nov., 1909	6 gallons	Slightly brackish	31.2	No houses near	0	0	0	0	1 or less	
Polo	Bulacan	do	Mar. 26	137	Feb., 1908	3.5 gallons	Brackish	30.0		0	0	0	0	2 or less	
Salinas	Cavite	Near schoolhouse	Mar. 14	115	Feb., 1910	Slow			No houses near	0	0	0	0	1 or less	
San Nicolas	Bulacan	Near church	Mar. 18	72	do	5 gallons	Sweet	28.6	Estero about 9 meters away	0	0	0	0	do	
Santa Cruz	Cavite	Salinas Street	Mar. 14	55	May, 1911	Medium			Houses about 9 meters away	0	0	0	0	do	
Do	do	Near schoolhouse	do	76	June, 1911				Houses about 12 meters away	0	0	0	0	do	

[a] Positive in 5 cc., but there was possibility of contamination of sample en route to laboratory.

[b] Not known.

INFANT MORTALITY IN THE PHILIPPINE ISLANDS

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Infant mortality in Manila is greater than it is in any other city from which we have records. This excessive mortality is not due to a single cause, and it is not due to natural conditions of the country. It is due to a multiplicity of artificial causes that may be classified into: Predisposing causes, prenatal and postnatal, and immediate or active causes.

A thorough study of the predisposing causes of infant mortality necessitates careful investigation of the mentality, financial responsibility, social and political economy of the people, the sanitary conditions—including character and quality of medical attendance—conditions of childbirth, general hygiene, personal hygiene, habits, vices, and customs of the race. In this connection, also, must be considered the influence of heredity, with particular reference to tuberculosis, syphilis, and other diseases transmitted directly or indirectly through generations—in other words, the eugenic estimate of the race.

Of the more direct influences bearing upon the prospects of the child after birth, there must be considered the environment, the character and method of feeding, and the influence of disease.

The committee for the investigation of infant mortality has been proceeding with its work along lines as indicated in the above outline, and a preliminary report of its findings has been submitted to the Philippine Legislature.

In the study of so complex a subject as is that of infant mortality, one must constantly bear in mind: First, careful attention to all details in order to secure facts, and, secondly, the necessity for constant attention in order to keep cause and effect in their proper relation to the question under discussion. A great many of the mistakes made in reports of students of this subject are in mistaking cause for effect and making recommendations in accordance therewith.

I shall only discuss, in the briefest possible way, a few of the most important questions involved in this great problem, and only the food situation for adults and children will be considered in this report.

FOOD SITUATION

The under-developed and under-nourished condition of the great masses of the Filipino people is due to a number of causes, the principal one being insufficient quantity and injudicious variety of foodstuffs employed. The cause of the enormous influence of the faulty nutrition of the mothers upon infant mortality, directly and indirectly, is one of the most important subjects within the scope of any investigation of this character. The small amount of data accumulated to date does not warrant definite conclusions, but a brief consideration of some of the facts is indicated.

Without going into the question of the much-discussed influence of special varieties of rice upon health and mortality, we may discuss the much larger question of the influence of metabolism disturbances, due to nutritional errors, upon infant mortality. As will be seen in another place in this report, the mortality in breast-fed children is higher than it is among children artificially fed. This condition, so far as we know, is peculiar to the Philippine Islands. The logical, and we believe the correct, explanation of this is the deficiency in quantity and quality of mothers' milk. So far as ordinary analysis shows the breast milk of Filipina mothers is of satisfactory quality for nutritional purposes. However, certain diseases (particularly infantile beriberi) are generally believed to be caused by some abnormality of mothers' milk. In a considerable number of cases studied from the clinics of the Philippine General Hospital, deficient quantity has been a rather constant finding. When these facts are considered, together with the under-nourished condition of the majority of the mothers due to the ravages of disease, we must conclude that faulty nutrition of the mothers is one of the principal factors in the enormous mortality of breast-fed children. The correction of this condition resolves itself into a discussion of methods for the improvement of the quantity and quality of mothers' milk and of the artificial feeding of babies.

In individual cases and to meet the immediate demands, satisfactory artificial feeding offers the obvious solution of the question. However, such a policy applied to the whole country would, eventually, lead to conditions more unwholesome than are those of the present time, and the ultimate solution of the problem, therefore, must depend upon improvement in the nutrition of the race. There are not in history more pathetic examples of unavailing self-sacrifice than are daily seen in our large clinics, of poor, half-starved, under-nourished mothers attempting to supply

from their breasts food for one or more children, when their own metabolisms are in a starved condition. When asked the direct question as to the supply of foodstuffs, these mothers almost invariably state that they have plenty to eat, and the pathetic part of the story is that they believe that they are stating facts. These abnormal premises are the result of a peculiar unexplainable psychology that is of very wide application in this country that the administration of food is more to satisfy hunger than to produce flesh and blood, and that the cheapest way in which hunger may be satisfied produces a satisfactory form of existence. It has been stated repeatedly that Filipinos do not care for foods other than fish and rice, with a few condiments and vegetables, but investigation tends to show that this is not a fact, and that these people have the same appetites and desire for fat and heat-producing foods as have people of other countries.

INFANT FEEDING

Good milk is the only satisfactory food during infancy. Mothers' milk, under normal conditions, is the ideal food, and next, because of its physiological adaptability and because it is the only class of milk it is possible to produce in quantities sufficient to meet the world's needs, is cows' milk. With the conditions discussed above, showing the causes for deficiency in the quantity of mothers' milk, together with the well-known fact that fresh, clean, raw, cows' milk is not obtainable in large quantities in the Philippine Islands, and that the prospect for a sufficient local production seems very remote, there is shown a new problem in infant feeding.

In considering the physiological requirements for the production of satisfactory baby food, it must be remembered that milk is just as essential an article of diet for the nursing mother in cases of breast feeding as it is for the baby in cases of artificial feeding, and recommendations for the solution of our local problem must bear this point in mind. The milk production of the Philippine Islands is practically nil when considered in relation to the requirements of the country. The principal supply consists of carabaos' milk and goats' milk, with a few dairies located in the larger cities, making a business of supplying cows' milk. We have gone rather carefully into the question of the quality of these milks, it being impossible in the time allowed to do anything regarding the correct estimation of the quantity produced. Nor is this necessary, because investigation of the quality leads to but one conclusion, and that is *that practically all fresh milk produced in this country is dangerous to health, in whatever man-*

ner used, and the marketing of these products should be interdicted by law. Carabaos' milk and goats' milk, when obtained from healthy clean animals, properly fed, and under proper sanitary surroundings, are excellent milks, but the requisite conditions do not obtain in the Philippine Islands, and with possibly one or two exceptions the conditions regarding the local supply of cows' milk are equally unsatisfactory. Nor is this all, for by no method of reasoning can we foresee a time when it will be practicable to produce satisfactory surroundings consistent with an ample supply of fresh milk *at a reasonable price.* The present custom of collecting, transporting, and using the local milk supply is unbelievably filthy, unsanitary, and consequently dangerous, and a continuance of the present practice with the facts before us should fix criminal responsibility for the loss of life. A general idea of the methods in vogue in collecting and marketing carabaos' milk is shown in a report by Doctors Abella and Gabriel. Briefly, the milk sold on the streets of Manila—and presumably in other cities as well—is from twenty-six to thirty hours old; has been diluted with tap water, or worse; has been collected and transported in dirty receptacles; has been milked by unclean persons from unclean animals; and both chemical and bacteriological examination, of course, shows this milk to be just about as bad as it is possible to make it. We have not seen a single sample that would even approach the margin of safety for its use by human beings, and in many instances evidences of sewage contamination and the presence of extremely dangerous bacteria are found in samples of milk bought in the open market. The same is true, to a less degree, of so-called fresh cows' milk sold in Manila. Under special conditions, which are obtained only at the expense of a very high cost of production, surroundings have been produced by which clean milk could be marketed. Notable in this respect is the very excellent work of La Gota de Leche which by careful supervision of model dairies has been able to produce good milk; but even under these circumstances, which raise the cost of milk to 50 centavos [1] a liter, the distinguished officials controlling the policy of this institution have felt it necessary to sterilize the milk before allowing its consumption by the babies under their care. If sterilization still is necessary after the precautions and expenses incident to the production of milk by La Gota de Leche, the problem of furnishing raw, fresh milk in quantities sufficient to influence

[1] One centavo equals $0.005, United States currency.

infant mortality in this Archipelago would appear to be one surrounded by impossible difficulties.

Taking all the evidence into consideration, a raw, fresh milk supply, sufficient to meet the absolute requirements of the country, does not seem to be within the bounds of possibility—at least within a reasonable length of time. All authorities acknowledge that raw milk contains elements of nutritional value not found in any sterilized milk, and so far as we are informed the only differences to be found between sterilized milk are differences in chemical composition. Therefore, in all probability, sterilized milk of local production has no advantage over imported sterilized milk. The question, then, resolves itself purely into one of financial consideration. Other things being equal, the cheapest milk should be the one adopted for our general use.

PASTEURIZATION

So much has been written recently regarding the methods of Pasteurization of milk in tropical countries that a very brief consideration of this subject seems pertinent. Formerly, Pasteurization was considered an efficient method of preparing milk for human consumption, because of the destruction by this method of dangerous disease-producing bacteria. We now know that the so-called pathogenic organisms are not the only, even if they are the most dangerous, bacteria in milk. Pasteurization, of course, does not destroy spore-bearing bacteria, and, therefore, any milk not kept below a temperature of from 20 to 22° C. after Pasteurization acts as a culture medium for those germs not destroyed by the low degree of heat used in the method of Pasteurization. Intrinsically, most of the bacteria of this class are not considered pathogenic, but as a result of their multiplication the chemical composition of the milk is altered, and as by-products of this alteration there are produced dangerous chemical poisons which are very important factors in the morbidity results produced by the ingestion of milk. Conditions for the growth of bacteria in the Philippine Islands are ideal, and with a very limited ice supply and without much prospect of improving this condition the after care of either fresh or Pasteurized milk becomes impossible for the vast majority of people. Actual experimentation has shown that the multiplication of bacteria in Pasteurized milk is so rapid that within a few hours after Pasteurization such milk is almost as dangerous as if this process had not been employed.

We come, then, to completely sterilized milk as being the only

variety of this life-giving food practicable of extensive employment in this country, at least at the present time.

Fortunately, conditions are not so bad as they would appear at first sight. Sterilized milk when used under proper conditions is a very satisfactory food for infants, and is just as satisfactory for all other purposes as is raw milk, and another fortunate circumstance is that the Philippine Islands enjoys a splendid market of imported sterilized, natural, and condensed milks of excellent quality at very reasonable prices; so that, as pointed out by me in an article presented to the Congress of Filipino Physicians, the milk supply of the Philippine Islands compares very favorably with that of many other countries and cities. It is a fact that sterilized milks are a little more indigestible than are raw milks, and there are certain metabolism conditions, for example, scurvy, that may be incurred as a result of the use of sterilized food. However, both the indigestibility and the metabolism-disturbing qualities of such milk are easily and satisfactorily controlled by simple methods well-known to the medical profession. These methods are so successful that in one series of records of more than 1,000 babies born in the Philippine Islands, and fed entirely on these sterilized foods, there has not been a single case of metabolism disturbance nor a death from disease of importance that could be justly attributed to the use of such food.

It may be of interest to note that there was imported into the Philippine Islands during the fiscal year 1912, an equivalent of between 18 and 20 million kilograms of milk, at an approximate valuation of 15,000,000 pesos.[2]

METHODS OF ARTIFICIAL FEEDING

The methods employed in the artificial feeding of infants among the poor people of Manila are faulty in many particulars. In the first place, notwithstanding the accessibility of a very good milk supply, the foods supplied to children in a majority of cases are those of condensed, sweetened, skimmed milk of the cheapest varieties, and consequently poor in quality. In another place I have discussed this subject at length.[3] In this report it was shown that the apparent economy in the use of this food, figured from a financial basis alone, is not a true economy, because milk compounds of this class contain from 50 to 65 per cent of ordinary sugar. When the caloric value of

[2] In United States currency, 7,500,000 dollars.

[3] Proceedings of the Congress of Filipino Physicians, held in Manila this year.

the actual milk contained in these tins is figured at the current prices, and this price subtracted from the total price of a tin of one of these mixtures, it is found that the people pay an average of from 50 to 75 centavos a kilogram for ordinary sugar, which they can buy in a *tienda* [4] for 11 centavos a kilogram.

It is, of course, unnecessary to dwell upon the undesirability of the use of this class of foods, and it only remains to point out that it is bad in principle, and what apparently, heretofore, has not been recognized that it is a more expensive method of feeding than would be necessary by the employment of good qualities of milk.

It should be stated that there is one favorable feature in the use of sugar-preserved milk compounds, and that is that the excessive amount of sugar preserves the food from the time of the opening of the tin until the food is entirely consumed. This is, of course, an important problem with poor people who cannot afford the ice necessary for the preservation of any pure milk, whether sterilized or not, after the tin is opened. However, this should not be a serious obstacle in the adoption of the use of a better grade of milk, because the method that is used to a greater or less extent among the poorer people of the United States, in which a number of neighbors who have nursing children alternate in the opening of the tins of food, so that each tin when opened is consumed by a number of babies in a few hours, might well be adopted here. Another solution of this problem that already is being employed by some manufacturers consists in marketing milk in much smaller tins.

The next most important faulty custom consists in the dilution of milk compounds with unsafe water. In our investigation of the causes of death of 300 babies, it is found that tap water, either with or without boiling, is used as a diluent in most instances. As a majority of the houses of these people are at considerable distances from the nearest faucet, the water is carted by water carriers and kept in earthenware jars or other vessels, under the most unsanitary conditions; in many instances whatever safety might be secured by boiling the water is destroyed by the subsequent manipulations and care of the water and by the methods employed in making the dilutions of the milk mixtures. The proof that these mixtures are dangerous to the health of the baby, on account of the introduction of bacteria, is shown by the analyses of the contents of a number of nursing bottles already prepared for consumption by the

[4] Small, native shop.

baby. These analyses of the finished product of food just before administration show dangerous contamination in practically every instance, and this has been found particularly true in cases of children dying from gastro-intestinal disturbances.

Other faulty methods which need not be discussed at length here are the almost universal custom of feeding babies with the greatest irregularity in time, quantity, and strength of food administered.

The remedy for these conditions, obviously, is education, both by theoretical instruction and, best of all, by practical demonstration as may be seen in the wards and clinics of the hospitals and La Gota de Leche, and, as has been recommended by the Committee for the investigation of Infant Mortality, by the establishment of nursery maids training schools and day nurseries.

The remedy controlling the character and quality of foodstuffs employed, however, lies in the hands of the legislative body, and in the opinion of the committee above referred to the question should be treated by discriminating high import duty on unsatisfactory milk compounds and by allowing free entry to the better qualities.

In order to solve the infant mortality question in this or any other country, the first essential is to secure the influence of a favorable and interested public opinion.

The attitude of public opinion in health matters is a very popular one, and even in older countries with more advanced civilization it is only within recent years that conservation of health has been of much interest to the general public.

Public opinion is vitally active regarding the pecuniary interests of a country, as exemplified in commercial activities and improvements, and even in the health and protection of draft animals and in the comfort and well-being and protection from cruelty to domestic animals.

However, with regard to the great vital question of the conservation of the health of its citizens and the saving and protection of the lives of infants, there exists a curious indifference that only springs, periodically, into activity as the result of some spectacular catastrophe, and dies down again with the restoration of the usual equilibrium.

The great Taal Volcano eruption destroyed some two thousand lives and a great deal of property, and its results sent waves of horror throughout the world. There are more lives uselessly sacrificed to tuberculosis in the Philippine Islands every month

than were destroyed by the Taal eruption; and the economic loss to the country by decrease in potential energy and earning capacity of its citizens, to say nothing of the actual loss of life, costs the country daily many times the value of property destroyed by Taal.

The recent catastrophe in Cebu and other southern islands sent a wave of horror over the country and called out Government and private reserves to meet the requirements of the situation. The loss of life and health is greater from criminal obstetrical practices in the Philippine Islands every day of the year than was the loss of life at Cebu. The financial drain upon the resources of the country as a result of these preventable and criminal practices is a greater daily drain than the total value of the property destroyed by this unavoidable calamity.

In older and more experienced countries there is at last an awakened public opinion regarding the economic consideration of health problems, and one of the most important questions for us is to secure the support of this valuable weapon in our campaign for the conservation of the lives of the potential citizens of this country.

THE PROTECTIVE POWER OF NORMAL HUMAN MILK AGAINST POLYNEURITIS GALLINARUM (BERIBERI)

By R. B. GIBSON

(From the Laboratory of Physiology, College of Medicine and Surgery, University of the Philippines)

Andrews [1] has pointed out in a recent paper that the cause of infantile beriberi, to which the high death rate of infants in Manila is in part attributable, is primarily due to the quality of the mothers' milk. The disease is not an infection or toxæmia of either the mother or child. Presumably the etiology is associated with a deficiency of the protective substances or "vitamines" [2] of the milk, induced by the too exclusive consumption of milled rice by the mother. The symptoms of the beriberi may not be apparent in the mother on the first examination, but usually appear later if the child continues nursing.

Probably with a deficiency (of the protective substances) in her diet the mother draws on her own storehouse for this substance for her child, thus diminishing her own supply and producing the disease in herself.

Doctor Andrews and I had planned to study the protective power of the milk of the mothers of infantile beriberi cases, the diagnosis of which could be verified by autopsy. However, I have not yet been able to obtain a case in which autopsy was permitted and which, at the same time, would supply sufficient milk for experimentation. The milk obtained was scant, and the secretion ceased in the course of a few days in the two instances where the autopsy was allowed and the diagnosis verified. Some control observations on the protective power of normal human milk have been made, and I have thought it worth while to present these in a short paper.

In view of the conclusive evidence that rice polyneuritis is a nutritional disease caused by dietetic deficiency, it seems hardly necessary to accept the presence of a toxic substance in the milk as suggested by Guerrero [3] from experiments on the frog heart. Analyses of the milk of women with beriberi infants, reported by Andrews, show that some of the samples are quite normal,

[1] *This Journal, Sec. B* (1912), 7, 67.
[2] *Cf.* Funk., *Journ. Physiol.* (1913), 46, 173, and earlier papers.
[3] *Bull. Manila Med. Soc.* (1912), 4, 167.

so far as proteid, fat, and carbohydrate are concerned; however, the amounts of phosphate and calcium are increased, the latter from three to four times the normal. The increased calcium, in itself, ought to be the cause of the early cessation of the heart's action in Guerrero's experiments.

Vedder and Clark [4] have reported experiments with normal cows' milk. Fowls fed on polished rice and receiving in addition 5 cubic centimeters of canned milk received little or no protection against polyneuritis gallinarum. With 5 cubic centimeters of fresh cows' milk, they received partial protection as indicated by the prolongation of the "incubation" period. It is to be expected that sterilized milk would not be as efficient as fresh cow's milk, in as much as Fraser and Stanton [5] have found that the protective vitamine may be destroyed by autoclaving.

The milk used in the present experiments was obtained from the obstetrical ward of the Philippine General Hospital. It was not obtained until three days after parturition. The women usually enter the ward previous to labor. They were, therefore, for several days on the diet given below before the milk was collected. The breasts were milked by hand into a sterile flask, mixed samples being obtained daily.

The diet was as follows:

Breakfast. Two eggs, 2 slices of bread, coffee, milk, and sugar.
Lunch and dinner. Fish or stewed beef, unpolished rice, cooked vegetables, pudding or banana, tea, and milk.

It was planned in the present experiments to feed 4 groups of 3 fowls each on (1) 50 grams of polished rice and 5 cubic centimeters of human milk, (2) 50 grams of polished rice and 10 cubic centimeters of human milk, (3) 50 grams of polished rice and 20 cubic centimeters of human milk, and (4) 50 grams of polished rice alone, per day. On some days it was impossible to get any milk because of the lack of suitable patients in the ward, and on other days it was necessary to cut down or omit the milk ration for one or more of the fowls receiving 20 cubic centimeters per day. Fortunately, the milk was obtained in abundance from the twenty-seventh to the fortieth day, inclusive, of the experiment. On the fortieth day 1 fowl (8) receiving 20 cubic centimeters of milk came down with mild though typical neuritis, the experiment indicating conclusively that the administration of 20 cubic centimeters of human milk with the polished

[4] *This Journal, Sec. B* (1912), 7, 423.

[5] Studies from Institute for Medical Research. Federated Malay States (1911), No. 12.

rice is insufficient to prevent the onset of neuritis. The experiments are given in the accompanying table.

It is evident from the results obtained that the almost continuous daily administration of 5 cubic centimeters (fowl 3) and 10 cubic centimeters (fowl 5) of human milk with the polished rice did not prevent polyneuritis. Furthermore, 20 cubic centimeters of the milk is insufficient as stated above. Clark has shown that degeneration of the nerves of fowls may be observed as early as the seventh day of continuous rice feeding; it would seem, then, that histological examination of the nerve on about the twentieth day of the experiment would be a more exact method of determining whether or not a certain substance is protective. For example, fowls 1, 7, and 10 (the control) have not developed neuritis in fifty-seven days. However, fowls 2, 6, 9, and 11 showed distinct degeneration when killed on the twentieth day. Finally it would seem, if Vedder's statement that fowls kept on 5 cubic centimeters of fresh cows' milk with milled rice are partially protected be accepted, that normal human milk must contain not more than one-fourth of the amount of the vitamine of the former.

TABLE I.—*Record of rice and milk fed fowls.*

Day.	Fowl 1.		Fowl 2.		Fowl 3.		Fowl 4.		Fowl 5.		Fowl 6.		Fowl 7.		Fowl 8.		Fowl 9.		Fowl 10.		Fowl 11.	
	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.	Milk fed.	Weight.
	cc.	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*	*cc.*	*Gms.*
1							10	1,407	10	1,239	10	1,399	20	1,085	20	1,289	20	1,299	0	1,307	0	1,204
2	0	1,450	0	1,425	0	1,292	0	1,414	0	1,230	0		0	1,095	0	1,294	0	1,254	0	1,315	0	1,199
3	0	1,430	0	1,427	0	1,285	0	1,409	0	1,235	0	1,417	0	1,097	0	1,309	0	1,274	0	1,334	0	1,210
4	5	1,394	5	1,414	5	1,264	10	1,397	10	1,195	10	1,410	20	1,095	20	1,280	20	1,255	0	1,310	0	1,212
5	5	1,365	5	1,427	5	1,232	10	1,407	10	1,157	10	1,402	20	1,105	20	1,302	20	1,270	0	1,304	0	1,174
6	5	1,412	5	1,414	5	1,200	10	1,390	10	1,149	10	1,410	20	1,119	20	1,289	20	1,264	0	1,324	0	1,172
7	5	1,412	5	1,394	5	1,212	10	1,392	10	1,199	10	1,412	20	1,119	20	1,287	20	1,252	0	1,327	0	1,175
8	5	1,419	5	1,380	5	1,226	10	1,385	10	1,187	10	1,407	20	1,122	0	1,282	0	1,315	0	1,315	0	1,172
9	5	1,429	5	1,419	5	1,229	10	1,389	10	1,200	10	1,434	10	1,114	20	1,285	20	1,250	0	1,335	0	1,187
10	5	1,427	5	1,405	5	1,229	10	1,385	a10	1,205	10	1,427	20	1,085	20	1,289	20	1,247	0	1,332	0	1,189
11	5	1,429	5	1,404	a5	1,234	10	1,392	10	1,205	a10	1,397	a20	1,099	20	1,282	20	1,247	0	1,322	0	1,189
12	5	1,435	5	1,407	5	1,230	10	1,395	10	1,235	10	1,404	20	1,122	a20	1,315	20	1,272	0	1,357	0	1,212
13	5	1,432	5	1,400	5	1,234	10	1,399	10	1,234	10	1,397	20	1,117	20	1,322	20	1,270	0	1,340	0	1,207
14	5	1,427	5	1,389	5	1,235	10	1,397	10	1,240	10	1,410	20	1,125	20	1,325	20	1,272	0	1,369	0	1,214
15	0	1,427	0	1,387	0	1,238	0	1,404	0	1,244	0	1,410	0	1,134	0	1,324	0	1,262	0	1,377	0	1,230
16	0	1,427	a0	1,342	0	1,245	0	1,410	0	1,240	0	1,399	0	1,119	0	1,319	0	1,254	0	1,382	0	1,224
17	a5	1,435	5	1,330	5	1,245	10	1,405	10	1,239	10	1,396	5	1,119	5	1,302	5	1,229	0	1,374	0	1,247
18	5	1,415	5	1,335	5	1,235	10	1,405	10	1,240	10	1,412	10	1,162	10	1,327	10	1,257	0	1,389	0	1,254
19	5	1,400	5	1,335	5	1,240	0	1,409	0	1,242	0	1,425	0	1,145	0	1,330	0	1,242	0	1,392		1,250
20	5	1,387	5	1,327	5	1,234	10	1,405	10	1,235		1,420	5	1,147	5	1,327		1,210	0	1,400	Killed; sciatic nerve showed moderate degeneration.	
21	0	1,415	Killed; sciatic nerve showed typical degeneration.		0	1,240	a0	1,430	0	1,244	Killed; sciatic nerve showed some degeneration.		0	1,149	0	1,322	Killed; sciatic nerve showed beginning degeneration.		0	1,390		
22	5	1,435				1,230	10	1,415	10	1,235			20	1,145	20	1,310			0	1,380		
23	5	1,410			Neuritis.		10	1,425	10	1,235			20	1,145	5	1,322			0	1,375		
24	0	1,395					0	1,430	0	1,235			0	1,150	0	1,317			0	1,405		
25	5	1,396					10	1,435	10	1,235			17	1,142	17	1,307			0	1,389		
26	0	1,385					0	1,425	0	1,250			0	1,157	0	1,307			0	1,382		

27	5	1,340			10	1,422	10	1,252		20	1,194	20	1,300		0	1,415	
28	5	1,354			10	1,417	10	1,267		20	1,189	20	1,304		0	1,410	
29	5	1,337			10	1,412	10	1,269		20	1,184	20	1,293		0	1,386	
30	5	1,302			10	1,404	10	1,272		20	1,199	20	1,305		*0	1,384	
31	5	1,325			10	1,420	10	1,260		20	1,212	20	1,305		0	1,369	
32	5	1,300			10	1,377	10	1,260		20	1,217	20	1,304		0	1,374	
33	5	1,275			10	1,367	10	1,255		20	1,225	20	1,304		0	1,372	
34	5	1,247			10	1,367	10	1,254		20	1,239	20	1,302		0	1,390	
35	5	1,297			10	1,369	Neuritis (?)			20	1,249	20	1,295		0	1,392	
36	5	1,265			10	1,347	on the 31st			20	1,254	20	1,294		0	1,399	
37	5	1,252			10	1,345	day; dy-			20	1,257	20	1,277		0	1,375	
38	5	1,244			10	1,334	ing on the			20	1,267	20	1,282		0	1,369	
39	5	1,240			10	1,335	35th; scia-			20	1,270	20	1,289		0	1,357	
40	5	1,270			10	1,375	tic nerve			20	1,275	20	1,282		0	1,377	
41	0	1,265			0	1,375	showed			0	1,270	0	1,282		0	1,362	
42	5	1,257			10	1,384	typical de-			17	1,267		1,284		0	1,352	
43	0	1,257			0	1,379	generation.			0	1,267	Neuritis,			0	1,242	
44	5	1,250			10	1,385				20	1,260	mild type.			0	1,337	
45	5	1,252			10	1,379				20	1,259				0	1,337	
46	0	1,247			0	1,379				0	1,264				0	1,337	
47	0	1,289			0	1,402				0	1,294				0	1,384	
48	0	1,277			0	1,372				0	1,272				0	1,372	
49	0	1,279			0	1,364				0	1,269				0	1,369	
50	0	1,270			0	1,350				0	1,264				0	1,360	
51	0	1,267			0	1,377				0	1,320				0	1,372	
52	0	1,265			0	1,387				0	1,297				0	1,364	
53	0	1,264			0	1,395				0	1,280				0	1,352	
54	0	1,264			0	1,394				0	1,259				0	1,354	
55	0	1,269				1,379				0	1,265				0	1,357	
56	0	1,274								0	1,260				0	1,352	
57	0	1,279								0	1,250				0	1,349	

* Fed by hand from this day on, so that the fowls always received 50 grams of rice daily.

PROTEOSES AND FEVER

By R. B. GIBSON

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Bordet [1] says:

Von Pirquet and especially Friedberger, have developed the theory that the phenomena of anaphylaxis play an essential part in the production of the symptoms observed in the course of contagious diseases. According to these scientists, the symptoms declare themselves when the antibodies, elaborated by the organism, begin to react with the microbes. This theory was based especially on the fact * * * that the simple contact of microbes with fresh serum may produce anaphylatoxin; this contact naturally takes place constantly in the infected organism.

Evidence of the relation of fever in infections to anaphylaxis is given in the work of Friedberger and Mita.[2] The injection of appropriate minute amounts of foreign protein into guinea pigs, sensitized to that protein, produces a marked transient rise in body temperature following the characteristic fall. The same results are observable with anaphylatoxin prepared in vitro and, also, with normal guinea pigs (although relatively greatly increased amounts of the protein must be employed in the latter case).[3] Successive injections yield temperature curves corresponding in type to those of infectious fevers.

In anaphylactic shock, the symptoms resemble the picture obtained for the intoxication resulting from the intravenous injection of proteoses (page 479). This similarity has been repeatedly pointed out.[4]

Anaphylactic-like shock in the normal organism is claimed

[1] *Journ. State Med.* (1913), 21, 459.

[2] *Zeitschr. f. Immunitätsforsch.*, Orig. (1911), 10, 216.

[3] Friedberger and Mita believe that anaphylaxis is an intensification of the ordinary reaction of the normal organism to foreign proteins. The shock following the injection of toxic normal sera has been similarly interpreted [Doerr and Moldovan, *Zeitschr. f. Immunitätsforsch.*, Orig. (1910), 7, 223].

[4] *Cf.* E. Zunz, *ibid.* (1913), 16, 581.

for tissue extracts,[5] for methyl guanidine,[6] for β-imidazolethylamine,[7] and for globin, histone, and protamine.[8] Anaphylactic symptoms have also been obtained with acetic acid, saponin, potassium cyanide, hirudin, metasilicic acid, colloid iron hydroxide, nucleic acid, and snake venoms;[9] with sodium oleate, oleic acid, morphine, codeine, and strophanthine;[10] and with salts of the heavy metals and tannin and phosphomolybdic acid.[11]

There is evidence, also, that the formation of anaphylatoxin is associated with the hydrolytic cleavage of the protein antigen.[12] Thus Pfeiffer says:

> Durch diese Trias von Beweisen, die Identifizierung des anaphylaktischen Reaktionkörpers mit dem Immunkörper, die Entstehung eines Giftes in vitro durch Hinzutritt des Komplementes zur Verbindung Eiweiss-antieiweiss und das specifische proteolytische Abbauvermögen der Serum anaphylaktischer Meerschweinchen dem Antigen der Vorbehandlung gegenüber, war es zum erstenmal sichergestellt, dass wir in der Erscheinung der Anaphylaxie nichts anderes vor uns haben als eine, parenteral sich abspielende, unter dem Freiwerden von Giften einhergehende Eiweissverdauung, die gerade durch ihre Lokalization in der Blutbahn von den deletären Folgeerscheinungen des anaphylaktischen Shocks gefolgt ist.

As already stated above, Friedberger and Mita have associated fever production with the anaphylactic symptom-complex. The close relationship of "peptone" intoxication to anaphylaxis is further suggested by the pyrexial action formerly ascribed to the proteoses. A summary of the literature on proteose fever may be given briefly.

Endo- and extra-cellular substances have been separated from bacteria. These substances withstand boiling, and are precipitated with alcohol or ammonium sulphate, their behavior in these respects resembling that of proteoses. When injected into healthy animals, these extracted substances incite fever,

[5] Popielski. See page 480.

[6] Heyde, *Zentralbl. f. Physiol.* (1911), 25, 441.

[7] Dale, *Journ. Physiol.* (1906), 34, 163; *ibid.* (1910), 41, 318; *ibid.* (1912), 43, 182.

[8] Schittenhelm und Weichardt, *Zeitschr. f. Immunitätsforsch.*, Orig. (1912), 14, 609.

[9] Doerr und Russ, *Zentralbl. f. Bakt. etc.*, Orig. (1912), 63, 241; Doerr und Moldovan, *loc. cit.*

[10] Friedberger und Moreschi, *Berl. klin. Wochenschr.* (1912), 49, 741.

[11] Szymanowski, *Zeitschr. f. Immunitätsforsch.*, Orig. (1912), 16, 1.

[12] Pfeiffer und Mita, *ibid.* (1909–1910), 4, 410; *ibid.* (1910), 6, 18; Abderhalden and coworkers, *Zeitschr. f. physiol. Chem.* (Hoppe-Seyler) (1909, 1910, 1911); Friedberger und Mita, Versammlung deutscher Naturforscher und Aerzte in Königsberg (1910); Pfeiffer, *Zeitschr. f. Immunitätsforsch.*, Orig. (1911), 10, 550.

and, in tubercular animals, show effects somewhat similar to tuberculin.[13]

Ott and Collmar [14] first noted that the introduction of proteoses intravenously produces a considerable and immediate rise in body temperature in rabbits. Matthes [15] found that the subcutaneous injections of proteoses (dentero-albumose of Neumeister) are more pyrogenic for tubercular than for sound animals. Krehl,[16] Krehl and Matthes,[17] and Rolly [18] report experiments in which pyrexial effects from proteoses were obtained. When prepared in the laboratory from protein material, however, the proteoses are neither so uniformly pyrogenic nor so pronounced in their effects as are the products of bacterial origin. Von Behring,[19] on the contrary, failed to observe any augmentation of temperature as the result of injecting sterile proteose solutions. Klemperer [20] does not consider the causative factor of fever to be proteose, but ascribes such effect to adherent impurities.

Considerable emphasis was laid by Krehl and Matthes [21] on the occurrence of proteoses in the urine in both infections and aseptic febrile conditions. Schultess [22] reached the same conclusion. Morawicz and Dietschy [23] have shown that the presence of proteoses in the urine is by no means constant in fever. Subsequently, Krehl retracted his ideas in regard to proteose fever.[24]

Other substances, injected or formed in the course of intermediate metabolism, have been reported as possessing more or less pyrogenic action. These include boiled or unheated enzymes;[25] various proteins, amines, amino-acids, and sodium salts;[26] pu-

[13] Krehl, *Arch. f. exp. Path. u. Pharm.* (1895), **35**, 222; Krehl und Matthes, *ibid.* (1895), **36**, 437; Centanni, *Deutsche med. Wochenschr.* (1894), **20**, 148, 176; Voges, *Zeitschr. f. Hyg. u. Infectionskrankh.* (1894), **17**, 474; Martin, Goulstonian lectures, *Brit. Med. Journ.* (1892), **1**, 641; Wood, *Lancet* (1896), **1**, 980.

[14] *Journ. Physiol.* (1887), **8**, 218.

[15] *Deutsches Arch. f. klin. Med.* (1894), **54**, 391.

[16] *Arch. f. exp. Path. u. Pharm.* (1895), **35**, 222.

[17] *Ibid.* (1896), **36**, 437; *ibid.* (1898), **40**, 434.

[18] *Deutsches Arch. f. klin. Med.* (1902), **78**, 250.

[19] Lehrbuch der allgemeinen Therapie, von Eulenberg und Samuel (1898–99), **3**, 991.

[20] Naturforscherversammlung, Kassel (1903), **2**, 67.

[21] *Arch. f. exp. Path. u. Pharm.* (1898), **40**, 430.

[22] *Deutsches Arch. f. klin. Med.* (1897), **58**, 325; *ibid.* (1898), **60**, 55.

[23] *Arch. f. exp. Path. u. Pharm.* (1905), **54**, 88.

[24] *Cf.* MacCallum, The Harvey Lectures. New York (1908–09), 55.

[25] Ott and Collmar, *loc. cit.*; Krehl, *loc. cit.*; Edelberg, *Arch. f. exp. Path. u. Pharm.* (1880), **12**, 283.

[26] Krehl, *loc. cit.*

rines and their precursors;[27] ammonia [28] and organic acids;[29] and sodium-halogen salts.[30]

Friedberger and Mita seize upon the possibility of the formation of pyrogenic proteoses to explain the temperature rise in sensitized guinea pigs referred to above. Thus they say:

> Wir müssen annehmen, dass die gewöhnliche Anaphylaxis dadurch zustande kommt, dass aus dem parenteral eingeführten Eiweiss Spaltprodukte entstehen, die tödlich wirken, und ebenso ist es bei der Darstellung des Anaphylatoxins in vitro.
>
> Genau wie wir nun hier durch Verringerung der eingeführten Menge zuerst statt Tod Temperatursturz, dann nach einer Dosis, die die Temperatur scheinbar unbeeinflusst lässt, Temperatursteigerung haben, bis wir schliesslich an einen unteren Schwellenwert kommen, genau so verhält es sich wenn in den präparierten Organismus nicht fertiges Anaphylatoxin, sondern Eiweiss in untertödlicher Dosis eingeführt wird.

It is inconceivable to me that 0.0000005 cubic centimeter of sheep's serum, as used in some of Friedberger and Mita's experiments, would yield sufficient proteose to produce pyrogenic effects, even though the animals be highly sensitized.

Further evidence, however, for a febrile reaction in anaphylaxis of causal relation to proteoses is given in a recent paper by E. Zunz.[31] This investigator studied the active and passive anaphylaxis for peptic proteoses, prepared from fibrin according to the methods of Adler, of Haslam, and of Pick, and for the Siegfried peptone from the same material. He finds that, if a third intravenous injection of heteroalbumose or protalbumose is given eight, twenty-five, or thirty days subsequent to the production of the anaphylactic shock, a rise of from 1° to 2° in the rectal temperature may be observed (although no effects were obtained for longer periods than thirty days).

The identity of "peptone" intoxication with anaphylactic shock has recently been questioned. Bordet believes that any such conclusion "is so far very unprecise." And, in as much as he [32] has shown that anaphylatoxin may be developed in vitro from

[27] Burian und Schur, *Arch. f. d. ges. Physiol.*, Bonn (1901), 87, 239; Mandel, *Am. Journ. Physiol.* (1904), 10, 453.

[28] Erben, *Zeitschr. f. Heilk.* (1904), 25, 33.

[29] Regnard, Combustions-Respiration (1879); Geppert, *Zeitschr. f. klin. Med.* (1880), 2, 356; Minkowski, *Arch. f. exp. Path. u. Pharm.* (1885), 19, 209; Kraus, *Zeitschr. f. Heilk.* (1889), 10, 1.

[30] Meyer, *Deutsche med. Wochenschr.* (1909), 35, 194; Friberger, *Arch. f. Kinderheilk.* (1910), 53, 17; Schloss, *Biochem. Zeitschr.* (1909), 18, 14; and others.

[31] *Loc cit.*

[32] *Loc cit.*

normal serum by the addition of agar, he concludes that "the theory of the production of poison by digestion and disintegration of the antigen does not therefore appear to be justified." Friedberger [33] claims that Bordet's results with agar are to be ascribed to admixed proteins, although the formation of anaphylatoxin with kaolin suspensions seems to have been earlier demonstrated.

Loewit [34] believes that there is insufficient evidence to show that "peptone" shock and anaphylactic shock are identical.

Eine grosse Reihe von mehr oder weniger shockartigen Vergiftungszustanden ist mit der akuten, aktiven anaphylaktischen Vergiftung nicht identisch. Hierher gehört die Pepton-, die β-Imidazoläthylamin und die Methylguanidinvergiftung. Hierher gehören ferner die Vergiftungen mit Essigsäure, Nucleinsäure, Kieselsäurehydrasol, Kupfersulfat, Sublimat, von welchen die drei ersten sofortigen Herztod bewirken.

Additional evidence that the symptoms of anaphylactic shock are not due to proteoses is given by Auer and Van Slyke.[35] These investigators examined, with the recent refined analytical methods, the amino-acid, peptone, and proteose content of the lungs of guinea pigs which showed undoubted anaphylactic shock. The analytical figures are essentially identical for the "shock" and the control animals.

A question of extreme importance to "peptone" intoxication is whether the symptoms are actually due to the proteoses injected or to the admixture of some substance physiologically very active. Continuing the work of earlier investigators, Chittenden and Mendel and their associates had established the fact that the rapid introduction of proteoses directly into the circulation would produce a marked fall in the blood pressure of the dog; other changes were an increased lymph flow and inhibition of the clotting powers of the blood, deep narcosis, and anuria. Pick and Spiro [36] concluded that these effects may be due to a contaminating substance, "peptozyme," of animal origin and soluble in alcohol. Underhill [37] took up the subject anew; employing native proteoses or those made from isolated plant proteins with vegetable enzymes, heat, or acid hydration, he obtained the "peptone" shock. Furthermore, he duplicated Pick and Spiro's inactive preparations, except for a more extended hydrolysis, and still observed the characteristic effects.

[33] *Zeitschr. f. Immunitätsforsch.*, Orig. (1913), 17, 323.
[34] *Arch. f. exp. Path. u. Pharm.* (1913), 73, 1.
[35] *Journ. Exp. Med.* (1913), 18, 210.
[36] *Zeitschr. f. physiol. Chem.* (Hoppe-Seyler) (1900), 31, 235.
[37] *Am. Journ. Physiol.* (1903), 9, 345.

Underhill's work, however, does not seem to have been considered by subsequent writers. Popielski [38] and his associates ascribe the active physiological principle of intestinal extracts and of Witte "peptone" to an alcohol-soluble substance "vasodilatine." In a recent paper, Popielski says:[39]

> Vasodilatin ist ein chemisch einheitliches Körper; es ist weder Cholin, noch β-Imidazoläthylamin, noch entsteht es durch Zerfall von Cholin.

The experiments, to be reported here, were carried out several years ago because of the suggestion by Krehl of the possibility of a contaminating substance being responsible for the pyrexial effects ascribed to the proteoses. The association of proteoses and of fever with the anaphylactic reaction has made them timely, and I have thought it worth while to present them in the present paper. The original problem was simply an inquiry as to whether pyrexial effects result from the injection of proteoses, prepared by gentle hydration from pure proteins without subsequent drastic treatment. Now, however, the results must be considered also in their relation to the subject of anaphylaxis.

TECHNIQUE OF THE EXPERIMENTS

A description of the proteoses used in the present experiments has been given in an earlier paper [40] on the pharmacological action of these products on the heart. The proteoses were made from four-times reprecipitated caseinogen, from recrystallized edestin, and from thoroughly washed pig fibrin, by digestion with a very active scale pepsin prepared by me. The proteoses were salted out with ammonium sulphate, redissolved, and again salted out. The salt was removed by dialysis for two weeks, with thymol as the preservative. The dialyzate filtrate was concentrated in vacuo as 50° to a sirup, precipitated with 95 per cent alcohol, and dehydrated with hot absolute alcohol. The mixed proteoses were obtained as a fine white powder which was easily soluble in water. The filtrates from the alcohol-precipitated proteoses were concentrated, precipitated with hot absolute alcohol, and, on drying, yielded a fine white powder, evidently proteose.

These alcohol-soluble proteoses may be prepared from Witte peptone or other mixed proteose preparations by extracting with

[38] *Arch. f. d. ges. Physiol.* (Pflüger) (1907), 120, 451; *ibid.* (1908), 121, 239; *ibid.* (1909), 126, 483; also Czubalski, *ibid.* (1908), 121, 395; Gizelt, *ibid.* (1908), 123, 540.

[39] *Zeitschr. f. Immunitätsforsch.*, Orig. (1913), 18, 562.

[40] Gibson and Shultz, *Journ. Pharm. & Exp. Therap.* (1909–10) 1.

hot 95 per cent alcohol. On standing in the cold the proteoses separate out as semicrystalline bodies, resembling the spherules which are intermediate stages in the change of the amorphous ovalbumin or serum albumin to the crystalline form. An ovalbumin alcohol-soluble proteose, so prepared, produced the typical fall in blood pressure, etc., when injected intravenously into a puppy; there was, however, no inhibition of blood clotting. (The experiment was not repeated for lack of sufficient material.)

Rabbits and guinea pigs were used as experimental animals.[41] The experiments were so conducted that the animals should receive the least possible handling during the series of observations. They were amply fed. Temperature readings (rectal) on a series of animals were always taken in the same order; in this way the time of the observation could be noted in the protocols under a single hour. In the guinea pig experiments, the proteoses (always dissolved in about 4 to 5 cubic centimeters of physiological saline) were sterilized at 100°; while for the subsequent observations the proteose solutions (injected dissolved in 10 cubic centimeters of saline) were passed through a Berkefeld filter. The subcutaneous injections were made with aseptic precautions.

Control observations were made on all the animals. The temperature regulatory mechanism in the guinea pig and rabbit is somewhat uncertain and unstable. The animals are heavily coated with fur; sweat glands are lacking or are few or rudimentary; and the cutaneous circulation, in the rabbit, at least so far as temperature regulation is concerned, is practically nil. Their small size offers relatively a greater body surface for loss of heat by radiation and conduction than obtains for the larger animals. Furthermore, the animals are timid and easily excitable, and environmental influences or handling may be reflected in the temperature readings. Individual variations are considerable, and diurnal changes are much greater and less constant than for man.[42]

[41] The experiments on guinea pigs were carried on in the Sheffield Laboratory of Physiological Chemistry, Yale University, in 1904. The observations on rabbits given here were made in 1906 in the Research Laboratory of the Department of Health of the City of New York with proteoses prepared at the Sheffield Laboratory.

[42] *Cf.* Pembrey, Shäfer's Text-book of Physiology. Young J. Pentland, Edinburgh & London (1898), 1, 790; also Simpson and Galbraith, *Journ. Physiol.* (1905), 33, 225.

EXPERIMENTS ON GUINEA PIGS

TABLE I.—*Experiment 10. Pepsin caseoses (alcohol soluble).*

Guinea pig; female; weight, 490 grams. Injection subcutaneously of 0.3 gram in physiological salt solution at 9.20 a. m. on March 8.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Mar. 3	39.3	38.4 to 39.6	8	No injection.
4	39.0	38.4 to 39.5	11	Do.
5	39.1	38.7 to 39.5	5	Do.
8	38.9	38.8 to 39.0	2	Before injection.
8	39.3	39.2 to 39.6	11	Proteoses, 0.3 gm.
9	39.1	38.8 to 39.5	6	No injection.

There are, apparently, no pyrexial effects to be noted in the above experiment. The temperature of the animal is at no observation higher after the injection than was observed at times in the controls.

TABLE II.—*Experiment 14. Caseoses.*

Guinea pig; female; weight, 590 grams. Injection of 0.5 gram of the mixed caseoses at 11 a. m. on March 25.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Mar. 23	39.2	38.7 to 39.4	6	No injection.
24	38.9	38.6 to 39.0	5	Do.
25	38.5	38.2 to 38.7	3	Before injection.
25	38.8	38.2 to 39.2	11	Proteose, 0.5 gm.
26	39.3	38.8 to 39.6	5	No injection.

In this experiment careful examination of the temperature variation shows that the proteose injection was without effect on the day of administration.

TABLE III.—*Experiment 22. Edestinoses (alcohol soluble).*

Guinea pig; female; weight, 600 grams. Received a subcutaneous injection of 4.5 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and on April 28, and of 0.4 gram of edestinoses at 12.30 p. m. on April 29.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 25	39.2	38.8 to 39.4	7	No injection.
26	39.1	38.9 to 39.3	7	Do.
27	38.9	38.9 to 39.0	2	Before injection.
27	38.9	38.6 to 39.2	7	Saline, 4.5 cc.
28	38.5	38.5 to 38.6	2	Before injection.
28	38.4	38.1 to 38.8	7	Saline, 4.5 cc.
29	38.1	37.7 to 38.4	4	Before injection.
29	38.9	38.5 to 39.2	7	Proteoses, 0.4 gm.
30		39.0	1	No injection.

When compared with the saline injection on April 27 and with the observations on April 25 and 26, it does not appear that the proteoses are pyrexial.

TABLE IV.—*Experiment 23. Caseoses (alcohol soluble).*

Guinea pig; male; weight, 440 grams. Injections of 4.5 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and on April 28, and of 0.45 gram of caseoses at 12.30 p. m. on April 29.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 25	39.4	38.7 to 39.8	7	No injection.
26	39.1	38.6 to 39.8	7	Do.
27	38.6	38.6 to 38.6	2	Before injection.
27	39.1	38.2 to 40.0	6	Saline, 4.5 cc.
28	37.9	37.8 to 38.0	2	Before injection.
28	38.6	37.9 to 39.0	6	Saline, 4.5 cc.
29	38.4	37.6 to 39.0	4	Before injection.
29	38.8	38.2 to 39.7	7	Proteose, 0.45 gm.
30		39.1	1	No injection.

The temperature changes in this guinea pig are very abrupt and erratic. A temperature rise (April 27) following the injection of the physiological salt solution is more marked than that noted subsequent to the administration of the proteose; similarly, higher normals were obtained for April 25 and 26, when no injections were made.

TABLE V.—*Experiment 24. Caseoses.*

Guinea pig; male; weight, 570 grams. Subcutaneous injection of 4.5 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and at 12 a. m. on April 28, and of 0.45 gram of caseoses in 4.5 cubic centimeters of salt solution at 12.30 p. m. on April 29.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 25	39.1	38.0 to 39.6	7	No injection.
26	38.9	38.6 to 39.5	7	Do.
27	38.7	38.6 to 38.8	2	Before injection.
27	38.9	38.4 to 39.4	7	Saline, 4.5 cc.
28	38.5	38.3 to 38.8	2	Before injection.
28	38.9	38.3 to 39.6	7	Saline, 4.5 cc.
29	38.6	38.0 to 39.2	4	Before injection.
29	38.9	38.0 to 40.0	7	Proteose, 0.45 gm.
30		38.4	1	No injection.

While in this experiment the caseoses produce a transient temperature rise to a figure slightly higher than any of the control observations, the pyrexial effects are not especially convincing. The control saline injections would possibly be called somewhat pyrogenic if the normal observations on the two days preceding were not in existence. As it is, the form of the curve of the variations on the two saline injection days is strikingly similar to the proteose day. The experiment demonstrates in a striking degree the danger of the misinterpretation of temperature observations in which the initial readings are taken as the normal for the guinea pig.

TABLE VI.—*Experiment 25. Edestinoses.*

Guinea pig; male; weight, 440 grams. Injections of 4.5 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and 28, and of 0.4 gram of edestinoses (in 4 cubic centimeters of salt solution at 12.30 p.m. on April 29.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 25	39.4	39.0 to 40.0	7	No injection.
26	38.9	38.4 to 39.3	7	Do.
27	38.9	38.9	2	Before injection.
27	38.7	38.3 to 39.0	7	Saline, 4.5 cc.
28	38.1	37.9 to 38.4	2	Before injection.
28	38.6	38.1 to 38.9	7	Saline, 4.5 cc.
29	38.5	38.4 to 38.6	2	Before injection.
29	39.0	38.0 to 39.7	7	Proteoses, 4.5 grams.
30		38.4	1	No injection.

The control injection records in this experiment show less variation than do the series of readings on the two previous normal days. The sudden jump of the record to 39°.7 within two hours of the proteose injection is

of interest. Higher figures, however, were obtained on the first normal control day, although not subsequently. Any pyrogenic effect, if manifested at all, in this experiment, must be interpreted as such from a consideration of the control saline injection figures alone.

TABLE VII.—*Experiment 26. Edestinoses (alcohol soluble).*

Guinea pig; male; weight, 550 grams. Injection of 4 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and 28, and of 0.4 gram of edestinoses (in 4 cubic centimeters of saline) at 12.30 p. m. on April 29.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 25	39.3	38.9 to 39.6	7	No injection.
26	38.8	38.7 to 39.0	7	Do.
27	38.8	38.7 to 38.9	2	Before injection.
27	38.8	38.4 to 39.0	7	Saline, 4 cc.
28	38.2	38.2 to 38.2	2	Before injection.
28	38.6	38.2 to 39.2	7	Saline, 4 cc.
29	38.7	38.5 to 38.8	4	Before injection.
29	39.5	38.5 to 40.2	7	Proteoses, 0.4 gm.
30		38.4	1	No injection.

The readings on the proteose day undoubtedly show a febrile reaction.

TABLE VIII.—*Experiment 31. Edestinoses.*

Guinea pig; female; weight, 600 grams; used previously in experiment 22. Injections of 5 cubic centimeters of physiological salt solution at 11 a. m. on May 4, and of 0.5 gram of proteoses in 5 cubic centimeters of salt solution at 11 a. m. on May 5.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
May 2	38.3	38.1 to 38.8	7	No injection.
3	38.0	37.7 to 38.4	7	Do.
4	38.1	37.9 to 38.3	3	Before injection.
4	38.5	38.3 to 38.8	6	Saline, 5 cc.
5	38.6	37.8 to 38.2	3	Before injection.
5	[a]38.7	36.1 to 39.2	9	Proteoses, 0.5 gm.
6		38.5	1	No injection.

[a] Omitting the one subnormal reading.

A rise to 39°.2 following the characteristic fall after the edestinose injection suggests some slight influence exerted by the proteoses; this rise, however, is so little marked that any pyrogenic effect is very doubtful. It may be due to the extra handling of the animal, for a larger number of readings were taken on this day than during the control periods.

TABLE IX.—*Experiment 32. Alcohol-soluble edestinoses and mixed edestinoses.*

Guinea pig; male; weight, 600 grams; used previously in experiment 24 on April 29. Injection of 5 cubic centimeters of physiological salt solution, 0.5 gram of alcohol-soluble edestinose, and 1 gram of mixed edestinoses at 11 a. m. on May 4, 5, and 6, respectively.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
May 2	38.2	37.7 to 38.8	7	No injection.
3	38.2	37.8 to 38.4	6	Do.
4	38.6	38.3 to 38.8	3	Before injection.
4	38.3	38.0 to 38.7	6	Saline, 5 cc.
5	38.2	38.3 to 38.6	3	Before injection.
5	38.6	38.2 to 39.2	9	Proteose, 0.5 gm.
6	38.0	37.8 to 38.3	3	Before injection.
6	38.0	34.9 to 39.0	12	Proteose, 0.5 gm.
7	37.9	37.5 to 38.2	3	No injection.

The temperature readings are much lower than in experiment 24. The subnormal temperature on May 6 is of interest. Pyrogenic effects are slight and transient if the effects can be interpreted as pyrexial.

TABLE X.—*Experiment 33. Alcohol-soluble caseoses and edestinoses.*

Guinea pig; female; weight, 500 grams. Injection of 5 cubic centimeters of physiological salt solution, 0.5 gram of alcohol-soluble caseoses, and 0.75 gram of edestinoses at 11 a. m. on May 4, 5, and 6, respectively.

Date.	Temperature.		Observations.	Remarks.
	Average.	Range of variation.		
	°C.	°C.		
Apr. 2	38.4	37.8 to 38.9	7	No injection.
3	38.2	37.8 to 38.5	6	Do.
4	38.7	38.0 to 39.1	3	Before injection.
4	38.6	38.2 to 38.8	6	Saline, 5 cc.
5	38.4	38.4 to 38.5	3	Before injection.
5	[a]39.0	36.9 to 39.2	9	Proteose 0.5 gm.
6	39.0	38.9 to 39.0	3	Before injection.
6	39.0	38.7 to 39.2	12	Proteose 0.75 gm.
7	38.3	38.1 to 38.6	3	No injection.

[a] Omitting the subnormal reading.

A questionable febrile rise of a fraction of a degree is noted as the result of the injection of the alcohol-soluble caseoses on April 5.

TABLE XI.—*Summary of experiments on guinea pigs.*

Proteoses.	Fever; experiment No.	Questionable; experiment No.	No fever; experiment No.
Edestinoses	26	25, 32, 33	31
Edestinoses (alcohol soluble)	----	32	22
Caseoses	24	----	14
Caseoses (alcohol soluble)	----	22, 23	10
Total (12)	2	6	4

Only 2, out of 12 experiments, have given a definite pyrogenic reaction. It would seem, then, that the above preparations are not consistently pyrexial for guinea pigs.

EXPERIMENTS ON RABBITS

In the following experiments, there is no evidence of fever as the result of the proteose injections. The protocols are given, therefore, without individual discussion.

TABLE XII.—*Experiment 46. Control saline injection.*

Rabbit; weight, 1,175 grams. Injected with 10 cubic centimeters of physiological salt solution at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°*C.*		*a. m.*	*p. m.*	°*C.*
Feb. 25	----	3.00	39.5	Feb. 27	12.00	----	39.1
25	----	7.00	40.1	27	----	2.30	40.0
26	9.00	----	39.5	27	----	6.00	39.9
26	----	12.30	39.5	27	----	8.45	40.1
26	----	7.00	39.9	27	----	11.45	40.1
27	9.15	----	39.4	28	9.00	----	39.4
27	10.15	----	(a)				

[a] Injection.

TABLE XIII.—*Experiment 47. Control.*

Rabbit; weight, 1,200 grams. Rabbit was handled exactly like the others, but its skin was merely punctured with the syringe needle on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°*C.*		*a. m.*	*p. m.*	°*C.*
Feb. 25	----	3.00	40.0	Feb. 27	12.00	----	39.4
25	----	7.00	40.0	27	----	2.30	39.5
26	9.15	----	39.4	27	----	6.00	40.1
26	----	12.30	39.5	27	----	9.00	39.5
26	----	7.30	40.2	27	----	11.45	39.8
27	9.15	----	39.1	29	9.00	----	39.4
27	10.15	----	(a)				

[a] Skin pricked.

TABLE XIV.—*Experiment 48. Edestinoses.*

Rabbit; weight, 1,100 grams. Injection of 0.6 gram of edestinose in 10 cubic centimeters of physiological salt solution at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°*C.*		*a. m.*	*p. m.*	°*C.*
Feb. 25	.....	3.00	39.5	Feb. 27	12.00	.....	39.4
25	.....	7.00	40.2	27	.....	2.30	40.0
26	9.15	.....	39.5	27	.....	6.00	39.9
26	.....	12.30	39.4	27	.....	9.00	39.8
26	.....	7.00	40.0	27	.....	11.45	39.7
27	9.15	.....	39.3	28	9.00	.....	39.4
27	10.15	.....	(a)				

[a] Injection.

TABLE XV.—*Experiment 49. Control injection.*

Rabbit; weight, 1,425 grams. Injection of 10 cubic centimeters of physiological salt solution at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°*C.*		*a. m.*	*p. m.*	°*C.*
Feb. 25	.....	3.00	39.5	Feb. 27	12.00	.....	38.4
25	.....	7.00	40.0	27	.....	2.30	40.2
26	9.15	.....	39.0	27	.....	6.00	40.0
26	.....	12.30	39.3	27	.....	9.00	39.6
26	.....	7.00	39.6	27	.....	11.45	39.5
27	9.15	.....	39.3	28	9.00	.....	39.3
27	10.15	.....	(a)				

[a] Injection.

TABLE XVI.—*Experiment 50. Edestinoses.*

Rabbit; weight, 1,450 grams. Injection of 1 gram of edestinoses at 10.15 p. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°*C.*		*a. m.*	*p. m.*	°*C.*
Feb. 25	.....	3.00	40.0	Feb. 27	10.15	.....	(a)
25	.....	7.00	39.9	27	12.00	.....	38.7
26	9.15	.....	39.5	27	.....	2.30	40.2
26	.....	12.30	39.5	27	.....	6.00	39.8
26	.....	7.00	40.0	27	.....	11.45	40.2
27	9.15	.....	39.4	28	9.00	.....	39.4

[a] Injection.

TABLE XVII.—*Experiment 51. Edestinoses (alcohol soluble).*

Rabbit; weight, 1,175 grams. Injection of 1 gram of edestinoses at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	------	3.00	40.2	Feb. 27	12.00	------	38.0
25	------	7.00	40.0	27	------	2.30	38.3
26	9.15	------	39.5	27	------	6.00	37.9
26	------	12.30	39.7	27	------	9.00	37.9
26	------	7.00	39.7	27	------	11.45	37.6
27	9.15	------	39.7	28	9.00	------	37.7
27	10.15	------	(a)				

[a] Injection.

TABLE XVIII.—*Experiment 52. Edestinoses.*

Rabbit; weight, 1,100 grams. Received 0.65 gram of edestinoses at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	------	3.00	40.0	Feb. 27	12.00	------	39.3
25	------	7.00	40.0	27	------	2.30	39.5
26	9.15	------	39.5	27	------	6.00	39.8
26	------	12.30	39.4	27	------	9.00	39.8
26	------	7.00	40.1	27	------	11.45	39.4
27	9.15	------	39.0	28	9.00	------	39.2
27	10.15	------	(a)				

[a] Injection.

TABLE XIX.—*Experiment 53. Caseoses.*

Rabbit; weight, 1,150 grams. Injection of 0.5 gram of caseoses at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	------	3.00	39.6	Feb. 27	12.00	------	38.9
25	------	7.00	39.7	27	------	2.30	39.9
26	9.15	------	39.6	27	------	6.00	39.9
26	------	12.30	39.4	27	------	9.00	40.0
26	------	7.00	39.9	27	------	11.45	39.8
27	9.15	------	39.4	28	9.00	------	39.8
27	10.15	------	(a)				

[a] Injection.

TABLE XX.—*Experiment 54. Caseoses (alcohol soluble).*

Rabbit; weight, 1,500 grams. Injections of 1.1 grams of caseoses at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	.....	3.00	40.2	Feb. 27	12.00	.....	40.2
25	.....	7.00	40.3	27	.....	2.30	40.0
26	9.15	.....	39.6	27	.....	6.00	40.1
26	.....	12.30	39.6	27	.....	9.00	40.1
26	.....	7.00	40.2	27	.....	11.45	40.0
27	9.15	.....	39.3	28	9.00	.....	39.4
27	10.15	.....	(a)				

[a] Injection.

TABLE XXI.—*Experiment 55, Fibrinoses.*

Rabbit; weight, 1,400 grams. Received an injection of 0.95 gram of fibrinose preparation at 10.15 a. m. on February 27. The animal gradually collapsed, dying about noon.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	.....	3.00	39.4	Feb. 26	.....	7.00	39.5
25	.....	7.00	39.8	27	9.15	.....	39.2
26	9.15	.....	39.4	27	10.15	.....	(a)
26	.....	12.30	39.3				

[a] Injection.

TABLE XXII.—*Experiment 56. Control saline injection.*

Rabbit; weight, 1,600 grams. Injected with 10 cubic centimeters of physiological salt solution at 10.15 a. m. on February 27.

Date.	Time.		Temperature.	Date.	Time.		Temperature.
	a. m.	*p. m.*	°C.		*a. m.*	*p. m.*	°C.
Feb. 25	.....	3.00	40.0	Feb. 27	12.00	.....	39.8
25	.....	7.00	40.2	27	.....	2.30	39.7
26	9.15	.....	39.5	27	.....	6.00	39.6
26	.....	12.00	39.4	27	.....	8.45	39.5
26	.....	7.00	40.1	27	.....	11.45	39.7
27	9.15	.....	39.3	28	9.15	.....	39.2
27	10.15	.....	(a)				

[a] Injection.

TABLE XXIII.—*Summary of experiments 46 to 56 inclusive.*

Proteose preparation.	Experiments.	Result.
Control physiological saline	3	No fever.
Control—no injection	1	Do.
Edestinoses	3	Do.
Edestinoses (alcohol soluble)	1	No fever. Temperature dropped below normal, and remained low.
Caseoses	1	No fever.
Caseoses (alcohol soluble)	1	Do.
Fibrinoses	1	Animal died as result of injection.

In the experiments with rabbits, the slight rise in temperature with some of the proteoses is insignificant and is amply accounted for by the control days and the saline-injected rabbits. In the earlier rabbit experiments, conducted at the Sheffield Laboratory, the results were not so uniformly nonpyrogenic. I believe that facility in handling the rabbits, gained from experience in the previous work, has been a considerable factor in promoting the uniformity of the experimental results in this last series.

From the evidence presented, then, it would seem that the primary cleavage products of pepsin-hydrochloric acid digestion, when prepared without drastic treatment, from purified and well-characterized proteins, never have more than a slight pyrogenic effect when injected subcutaneously into rabbits and guinea pigs. Any temperature rise, if present, is insufficiently pronounced to permit a direct inciting rôle to be ascribed to such proteoses in the production of the severe naturally occurring fevers.

ALBINISM IN THE PHILIPPINE ISLANDS [1]

By VICTOR G. HEISER and RAFAEL VILLAFRANCA
(From the Bureau of Health, Manila, P. I.)

One plate

So far as known, the first investigation of albinism in the Philippine Islands is that which was undertaken by the Bureau of Health.[2] Later Villafranca, while district health officer for Bohol Province, undertook the study of this condition among Filipinos. These investigations are embodied in a statistical report of 198 new cases.

It is evident that all people who appear to be partial albinos are not necessarily albinos, although there are many incomplete and imperfect cases among individuals who have red, yellow, yellowish, or auburn hair. The majority of such cases are probably modified albinism. But as all of them are included in most of the estimates, it is evident why South America, the Philippines, and other places where dark complexions predominate are said to furnish more cases of albinism than other regions.

In the early report mention was made of some of the peculiar views entertained by Filipinos as to the cause of albinism. Scientific men are just as far apart in their views as to the cause of this condition. Some state that the albinos once constituted a separate race and that the cases that are characteristic are atavistic in character. Blumenbach,[3] Winterbottom,[3] Sprengel,[3] and Otto [3] considered albinism to be a disease or the result of disease. Buffon explained its existence on the theory of shipwreck or the abandonment of Europeans, whose offspring resulting from union with the local inhabitants retained some of the original characteristics of their white ancestors. Later this

[1] With the exception of the republished portion of this paper, taken from the Annual Report of the Director of Health for the fiscal year ended June 30, 1910, this paper is from a report on albinism in the Philippine Islands prepared by Rafael Villafranca, Bureau of Health, Manila, P. I.

[2] Heiser, *Annual Rep. P. I. Bur. Hlth.* (1909), 51–53.

[3] Ciencias Médicas Diccionario Enciclopédico Hispano-Americano. Barcelona, Montaner y Simón (1887–1899), 26 vols. 1, 800.

theory was renounced by Buffon.[4] Lecat [4] attributes it to the influence of heat; Mansfield [4] and others to maternal impressions. The latter theory is very common in the Philippines, as is also the theory that it is due to the morbid imagination of the mother during the period of pregnancy.

The consensus of opinion now seems to be that the condition is due to faulty development of the pigment-producing apparatus.

According to Geoffroy Saint-Hilaire,[4] three varieties of albinism exist: the complete form, in which the pigmentary matter is entirely lacking; the partial form, in which the pigment exists in some places and is absent in others; and the incomplete form, in which pigment exists in all parts, but in quantity below the normal.

In nearly all cases albinism is congenital. However, partial albinism may be accidental; but no case has been recorded of complete albinism being other than congenital.

Doctor Montinola, district health officer for the Province of Occidental Negros, who has given the subject considerable study, believes that the disease is of neuropathic origin and that in all cases the condition can be referred to defects in the nervous constitution of the ancestors.

Doctor Hurley, district health officer for Iloilo Province, collected 10 cases in his district, in which he believed that consanguinity was an important factor. In some cases the relationship was so close as to include the parents in the same family.

In complexion albinos are white, yellowish, red, or reddish. Their eyes are blue, and in complete albinos the globe of the eye is entirely deprived of pigment. In Bohol, albinos are usually shortsighted and fear the light, and in many cases there is some bodily deformity. If the eyelids of these people are opened, rapid oscillations of the eyeball take place. Such cases exist elsewhere, but more particularly in this island.

There is a tradition among these people to the effect that many centuries ago a white race lived in the mountains, and that the present albinos are the atavistic descendants of some members of this race who intermarried with the dark-skinned natives.

It is a common belief that albinos are mentally subnormal. This is true only in so far as degeneration has influenced their physical condition. In albinos with healthy bodies no intellectual inferiority has been noticed, and cases of weak mentality which have been observed could be accounted for by some organic defect.

[4] Ciencias Médicas, etc., see footnote 3.

Livingstone,[5] states that in certain parts of Africa albinos were destroyed because they were looked upon as the omens of evil. In other countries they are worshiped as favored beings. Neither of these beliefs is entertained in the Philippines.

Doctor Llora, district health officer for Leyte Province, records a superstition that albinism is caused by an evil spirit called *cahoy-non*, who resides in the field and exerts his evil influence on persons who incur his displeasure. This belief is confined to the lower class. Albinos are neither hated nor admired; they are simply looked upon as having incurred the displeasure of this spirit for which there is no remedy.

Defects of vision are not constant. Eighteen cases of photophobia, 13 cases of nystagmus, and 3 cases of myopia are recorded in the report submitted. These are the cases that are usually called moon-eyed. Properly adjusted glasses will remedy the cases of myopia as it will in other people.

According to many observers and travelers, albinism is to be found among all the races and in every zone of the earth. It is known by different names in different countries; for instance, albinia, acromia congénita, and leucopatia. In the Philippines, also, albinism has different names according to the dialects of the provinces in which it is found. In the Visayan dialect of Leyte Province it is called *ila*, a word which signifies "marked;" others call it *pamusag*, which signifies "whitened" or "painted white." In Misamis it is called vulgarly *kabang*, when it is incomplete, and *linakaran sa buan* when it is complete. In Albay Province it is called *akos sin adlao*, which signifies "son of the sun;" in Zambales, *anac auló ó labang;* in Ilocos Sur, *ampurao.*

Albinism is found in the lower animals and even in vegetables. Villafranca has seen carabaos, pigs, and rats that were complete albinos. Doctor Garcia, district health officer for Zambales, describes, in his report on albinism, an American albino sow which gave birth to 9 pigs, 5 almost complete and 4 partial albinos. In the almost complete albino pigs the skin was transparent and of a pinkish white color, the hair was black, and the eyes blue. On the other hand, the partial albino pigs suffered from lack of pigment in certain parts of the skin and none was found in the hair of the body.

The first report on this subject by the Bureau of Health is as follows:

[5] Ciencias Médicas, etc., see footnote 3.

ALBINISM IN THE PHILIPPINE ISLANDS.[4]

At the instance of Dr. H. Fraser of the Institute for Medical Research, Kaula Lumper [Kuala Lumpur], Federated Malay States, Dr. C. H. Usher, of Aberdeen, Scotland, and Prof. Frederick Starr, of Chicago University, this office issued on April 28, 1908, the following circular, addressed to the medical inspectors and district health officers of this Bureau:

In view of the general interest in the question of albinism, information is respectfully requested as to whether albinos have come under your observation, and if so, you are respectfully requested to furnish this office without delay answers to the following questions:

1. The pedigrees of families in which one or more cases of albinism have occurred. The more extensive such pedigrees are the better.

2. All information is desired bearing on whether albinism is or is not the expression of a prevalence of scanty pigmentation in a particular stock. Hence particulars are desired as to color of hair and eyes, fecundity, general physical and mental vigor, and the occurrence in albinotic families of any other defects than albinism.

3. The influence of cousin marriages is of great importance to be carefully followed up.

4. Incomplete family records and particulars of single cases of albinism will also be useful and welcome.

5. Photographs of albinos will be valued, especially albinos of dark races.

6. Incomplete or partial albinism; instances of pied albinism are desired.

The investigators venture to ask whether you will kindly aid the research by sending particulars of any cases. Whilst the information itself will be treated as confidential, full acknowledgment of its source will be made when the subject comes to publication.

Incomplete notes often contain useful information and will be welcome when full records can not be obtained.

* * * * * * *

With the [above] circular there was sent a leaflet prepared by Dr. C. H. Usher, containing information as to the prevalence of albinism and a form for making reports, as follows:

ALBINISM.

Albinism occurs among all races, even the darkest. It appears to be frequent among Malayan peoples. I desire to secure specific information regarding all possible cases. The following will help to render observation definite. When impossible to make a full report, give what you can. The first three items are indispensable.

Report on case of albinism.

Name of subject.
Residence.
Race or tribe.
Hair; color; quality; secure a sample if possible.
Skin; color; quality; blushing? sunburn?
Eyes; color; movement; squinting? myopic?
Carefully draw the iris and color to show pigment distribution, etc.

[4] *Annual Rep. P. I. Bur. Hlth.* (1909), 51–53.

Disposition and character. Ability in different directions; deficiency in different directions.

Occurrence. Is the case sporadic? If not, give all possible information regarding similar occurrences in the family. Are the parents related? Name all the children in the family in order, marking the cases.

What is the native word for an albino? What is its literal meaning?

What, if any, popular ideas regarding albinos? What do "the people say" about them?

Secure photograph of the subject; where possible, two views—one square front, the other exact profile. [End leaflet.]

As a result of these circulars, forty-five cases of albinos were reported from seven provinces; Albay, 2; Bohol, 11; Ambos Camarines, 5; Ilocos Sur, 5; Manila, 1; Pampanga, 16; Tarlac, 5.

It is not claimed that the figures presented are correct or approximately correct. It is not reasonable to suppose that on the island Province of Bohol, with a population of 269,223, there are 11 albinos; while in the near-by island Province of Cebu with a population of 653,729 there is not a single albino, though it is probably true that albinism is more prevalent in Bohol than in other provinces, as there is more "folk-lore" concerning the condition. The Bohol term for albino is "bulao" from the Visayan word "bulauan" which means gold. Albinos with blond hair and dark skin are called "bugao" (yellow) and those who are entirely white are known as "uguis" (decolorized). In this province there is a tradition of a white people known as Taguibanua (cave dwellers) who once lived in the mountain caves of the island, and the popular belief is that albinos are the result of the mingling of these cave dwellers with the natives.

By some of the inhabitants it is believed that a few of the Taguibanua still exist, and, that whenever one is seen by a pregnant woman, an albino child is the result. This latter theory is accepted in the Province of Albay where there also exists a tradition of an ancient white race.

Another theory that prevails in both of these provinces, and more or less in all other provinces, is that albinism is due to some peculiar phase of the moon at the moment of conception.

In the provinces around Manila an albino is known as "anak arao," "child of the sun," from the belief that the mothers of albino children during pregnancy develop a "fancy" for gazing at the sun. This theory is also prevalent to some extent in all parts of the Philippines.

The accompanying table of "Albinism in the Philippine Islands" is presented as evidence of good faith and as a token that this office will continue the investigation of this interesting subject until it can publish a reliable table of albinism in the Philippine Islands.

ILLUSTRATIONS

PLATE I

FIG. 1. Male complete albino of Dimiao, Bohol.
2. Female complete albino of Manila, Luzon.

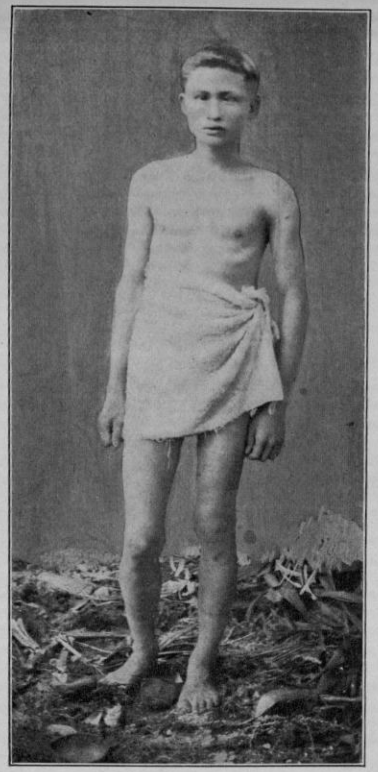

Fig. 1. Male complete albino of Dimiao, Bohol.

Fig. 2. Female complete albino of Manila, Luzon.

PLATE I.

TABLE I.—*Albinism among Filipinos in the Philippine Islands.*[a]

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														
1	Presentacion Balana	Female					Libog, Albay	White	Deep yellow	Pink	Pink	Pink					
2	Vicente Balivado	Male	7			Total	do	Absolute white	White	do	do	do	Excellent physique and health.				Daylight disagreeable, sight best after dark.
3	Luis Madrea	do	17	Single	Farmer	do	Valencia, Bohol	Thin, delicate, pink, and white.	Blond decolorated	Light blue	Golden	Dark	Weak and lymphatic, effeminate.	Poor	His mother's mother, great grandparents, 3 brothers, a sister, and other relatives.	3 brothers	Not shortsighted, feminine voice, eyes normal, marked double strabismus.
4	Dalmacia Madrea	Female					do	do	Coarse, blond brownish, unlustrous.	do	do	do	Weak	do	Grandmother, great grandparents, 3 brothers, a sister, and other relatives.	do	Eyes normal, strabismus, not shortsighted.
5	Francisco Namucot	Male	65	Married	Farmer	Partial	Dimiao, Bohol	Coarse, sunburnt, white in certain regions.	Black, ashy	Gold-brown	Brown and imperceptible.	Light blue	Weak, easily angered.	Average	Father, great grandparents, and 2 children.	2 daughters	Eyes normal.
6	Saturnino Goloso	do	24	Single	do	do	Tarbilaran, Bohol	Malay	Coarse, corn auburn	do	do	Blue	Strong, content, and happy.	Poor	1 sister and a grandfather.		Do.
7	Jacoba Madronero	Female	35		Weaver	do	Dimiao, Bohol	Sunburnt, white in parts, coarse, thin.	Brunette, slightly brown.	Light blue	Light blue	Dark	Strong and healthy, irascible.	Fair	All grandparents, 1 sister, and 1 daughter.	7 children	Do.
8	Paula Madrea	do	26	Married	None	Total	Valencia, Bohol	Pink, delicate, thin, and white.	Gold, brownish, unlustrous.	Blue	Blue	do	Fair	Poor		3 children	Eyes normal, fecund woman, all labors normal.
9	Julian Loresca	Male	24	do	do	Partial	Panglao, Bohol	Sunburnt, coarse, thin.	Auburn-brownish	Brown	Brown	Black	Sickly, weak, and nervous.	do	Father, father's parents, great grandparents, and 7 brothers.	9 brothers	Eyes normal.
10	Julian Lorica	do					do		Auburn, fine								
11	Pedro Lorica	do					do		Brown								
12	Ana Bongulto	Female					do		Old copper								
13	Maximino Busalanan	Male					Carmen, Bohol		Auburn at end, dark brown.								
14	Fabian Villareal	do	29	Married		Partial	Iriga, Camarines	White in chest and other regions.	Black, small blond patch in frontal region.	Brown	Brown	Black					No relationship between parents.
15	Luis Tizon	do	30	Single	Hemp stripper	Total	Lagonoy, Camarines	Pink-white	Cream-white	Pink	Pink	Pink	Good natured, robust, good worker.	Intelligent	No history of albinism in family.	All relations	Parents not related.
16	Vivencio Tizon	do	20	do	do	do	do	do	do	do	do	do	do	do	do	do	
17	Negrita No. 1	Female	9	do		do	Paracale, Camarines	White	Yellowish white		Reddish brown			Dull and apathetic.			
18	Negrita No. 2	do	11	do		do	do	do	do		do			Low			
19	Marciana Serrano	do	30			do	Cabugao, Ilocos Sur	Dark pink	Almost white, light straw.	Dark	Yellow	Dark		Good	1 aunt and 2 sisters	Parents and 5 brothers.	The Serrano family, residence Maradodon, Cabugao, Ilocos Sur, when all were living consisted of mother, father, 2 boys, and 3 girls, who were not albinos, and 1 boy and 2 girls who were albinos. Of the 2 boys who are not albinos, both are living, while the 3 nonalbino girls are dead. Of the 3 albino children, the boy is dead. Of the albino children, 33⅓ per cent are dead, while of the nonalbinos 60 per cent are dead, from which it may be assumed that albinism did not affect their physical condition. The 2 albino sisters are marked about equally; hair is of extremely light straw color, almost white. No history of albinism on either side. Parents not related. Mother is said to have been very fond of white flowers during pregnancy. Both albinos rendered uncomfortable by light.
20	Gregoria Serrano	do	25			do	do	do	do	do	do	do		do	do	do	

[a] From the Annual Report of the Director of Health for the fiscal year ending June 30, 1910. This table has been slightly changed for the sake of uniformity. [Editor.]

TABLE I.—*Albinism among Filipinos in the Philippine Islands*—Continued.

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other member of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														The Cabasug family of the barrio of Pila, Cabugao, Ilocos Sur, consists of father, mother, and 1 son not albinos, and 3 sons who are albinos, all living. These albino children resemble European children. Parents are fourth cousins. Mother twice married and had albino child (now dead) by first husband. Father has unpigmented birthmark, 1.5 square cm. in extent, just below left outer malleolus. Mother has jet black hair with exception of brown strands in left temporal region. These albinos seem uncomfortable in the light.
21	Sotero Cabasug	Male	11	Single		Total	Cabugao, Ilocos Sur	Fine blond	Light brown	Grayish brown	Grayish brown	Dark			1 brother and 1 sister	Parents and 1 brother.	
22	Alberto Cabasug	do	6	do		do	do	do	do	do	do	do			do	do	
23	Getrudis Cabasug	Female	4	do		do	do	do	do	do	do	do			2 brothers	do	
24	Petra Parcotelo	do	26	do		do	Manila	White	Blond	Blue	Blue	do	Vigorous	Vigorous			Parents dark and not related. From Iloilo. Observed by Doctor Catanjal in 1894.
25	Name not reported	do				Partial	Apalit, Pampanga										Observed by Doctor Catanjal in 1902.
26	do	do				Total	Tarlac, Tarlac										Has photophobia, "nictalopia," and nystagmus.
27	Florentina Cunanan	do	5			do	Angeles, Pampanga	White-pinkish	White and shiny	Pink	Pink	Pink	Delicate		Father and grandfather of mother, mother's sister, also 4 brothers.		
28	Mother of Florentina Cunanan.	do	35	Widow		Partial	do	Light					Delicate, frail, weak.				White semicircle surrounding upper part of both corneas.
29	Brother of Florentina Cunanan.	Male	10			Total	do	White, thin	White and fine								Toenails blackish.
30	Maura Toñgio	Female	40	Married		do	Bacolor, Pampanga	Blushing	Blond	Blue	Blue	Dark	Good habits and healthy.		3 children and 1 cousin	7 children	Chinese mestiza.
31	Ceferino Valdez	Male	22			Partial	do		Black						3 sisters and 1 aunt	7 brothers	
32	Lorenza Valdez	Female	16	Single		Total	do	Blushing	Brown-ocher	Light blue	Light blue	Dark			1 brother, 2 sisters, and 1 aunt.	do	Children of Maura Toñgio; 1 albino sister dead; great grandmother of father albino.
33	Eufrosina Valdez	do	10	do		do	do	do	Golden brown	do	do	do			do	do	
34	Benito Gozun	Male	12	do		do	do	Pale and dead white.	Semiauburn	do	do	do			1 sister, 1 brother, and 1 aunt.	5 brothers	Mother is cousin of Maura Toñgio, not albino; father not an albino.
35	Francisco Gozun	do	4	do		do	do	do	Dark gold	do	do	do			do	do	
36	Guadalupe Gozun	Female	[a]7	do		do	do	do	Gold, decolorated	do	do	do			2 brothers and 1 aunt	do	
37	Juan Varon	Male	46	Married		do	San Fernando, Pampanga.	Albino characteristic, very white.	Gold-blond	do	do	do	Good habits, good physique.		Nothing known	2 children	One of grandparents English mestizo.
38	Eugenia Mendoza	Female	50	Single			Guagua, Pampanga								Only case in family		
39	Apolinaria Castro	do	38	do		Total	San Fernando, Pampanga.	Blushing	Pale blond	Pink	Pink	Red				Parents	Orbits continually moving laterally. Born very small with unperforated anus; photophobia. Great grandfather English mestizo.
40	Eustaquia Alarcon	do	8	do		do	Candaba, Pampanga	White	Golden	Blue	Blue	Dark	Good	Defective memory.*	2 brothers	do	Brothers both myops. Had an albino brother who is dead. No consanguinity between ancestors.
41	Nicolas Alarcon	Male	5	do		do	do	do	do	do	do	do	do	Good	1 sister and 1 brother	do	
42	No name given	do					Moncada, Tarlac	Sunburn white	Blond	Brick-red	Brick-red	do		Less than average.	Yes		
43	do	do					do	do	do	do	do	do		do	do		Have been albinos for 3 generations, originally came from Badoc, Ilocos Norte.
44	do	Female					do	do	do	do	do	do		do	do		Parents were cousins.
45	Agustina Felicitas	do	52	Married	Shopkeeper	Total	Camiling, Tarlac	do		Blue	Blue	do	Weak and nervous.	Good	1 brother	1 daughter	

[a] Months.

TABLE II.—*Albinism among Filipinos in the Philippine Islands.*[a]

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														
1	Maria Fortón	Female	20	Single	Doorkeeper	Total	Santa Fe, Agusan	White, slightly reddish.	White, slightly golden.	Gray	Gray	Reddish	Strong	Feeble	Maternal grandfather's son.	6 brothers	Maria Fortón is a sister of Magdalena Fortón.
2	Magdalena Fortón	do	10	Child	do	do	do	do	do	do	do	do	do	do	do	do	Magdalena Fortón is a sister of Maria Fortón.
3	Claudia Goy	do	15	Single	do	do	Esperanza, Agusan	do	do	do	do	do	do	do	None	do	
4	Abundio Gomez	Male	1[b]	Child	None	do	San Luis, Agusan	do	do	do	do	do			Sister of maternal uncle	3 brothers	
5	Venancio Molina	do	17	Single	Journeyman		Daet, Ambos Camarines						Robust	Normal	None	2 brothers	
6	Dionicio Urcia	do	6	Child	None	Partial	Nasugbu, Batangas	White	Black	Pale	Gray	Black	do	do	do	do	
7	Juan Urcia	do	4	do	do	do	do	do	Light red	do	do	do	do	do	do	9 brothers	She shades her eyes when exposed to strong sunlight, and lids and lashes moved rapidly (photophobia and nystagmus).
8	Geronima Limbo	Female	70	Single	Weaver	Total	San Jose, Batangas	do	White	White with red spots.	do	White	do	do	do	None	
9	Maria Vergara	do	28	do	Spinner	do	Lemery, Batangas	do	Reddish	Light brown	do	do	Regular	do	do	None	
10	Ulderica Calapate	do	19	do	do	do	do	do	do	do	do	Reddish	do	do	do	do	
11	Perpetuo Sanchez	Male	29	do	Student	do	do	do	do	do	do	do	Normal	do	do	do	
12	Feliza Arandia	Female	29	Married	Spinner	do	do	do	do	do	do	do	do	do	Father and children	do	
13	Cresenciana Godoy	do	2	Child	None	do	Lobo, Batangas	Red spots	do	White	do	do	do	Regular	Maternal aunt	1 brother	
14	Monica Matibay	do	1	do	do	do	do	do	do	Light brown	do	do	do		None	3 brothers	
15	Victorio Lasala	Male	25	Married	Student	do	Taal, Batangas	White	do	Blue	Blue	Blue	do	Normal	1 daughter	None	
16	Bienvenida Lasala	Female	1	Child	None	do	do	do	do	do	do	do	do	do	Father	do	
17	Cresencia Lontoc	do	43	Married	Weaver	do	do	do	do	do	do	do	do	do	1 brother and 2 cousins	1 brother	
18	Filemón Mendoza	Male	7	Child	Student	do	do	do	do	do	do	do	do	do	None	None	
19	Miguela Mendoza	Female	36	Single	Servant	do	do	do	do	do	do	do	do	do	3 brothers	3 brothers	
20	Paterna Villanueva	do	53	do	Weaver	do	do	do	do	do	do	do	do	do	Mother, cousins, and 3 brothers.	5 brothers	
21	Leoncio Capuno	Male	33	Married	Laborer	do	Alitagtag, Batangas	Yellow	Brown	Brown		Black	do	Regular	None	1 brother and 3 children.	Four brothers (dead) not albinos.
22	Mamerto Casalla	do	26	Widower	do	do	do	do	Golden	do	White	do	do	do	do	2 brothers and 2 children.	One daughter dead, not an albino; 1 brother, albino, Tacloban, Leyte, 10 years after leaving this place.
23	Simplicio Abusman	do	11	Child	Student	do	Bauan, Batangas	do	do	White	Reddish	do	do	do	do	3 brothers	Photophobia.
24	Sebastian Azucena	do	27	Married	Laborer	do	do	do	Black	do	do	do	do	do	2 brothers	6 brothers and 2 children.	Do.
25	Felipa Azucena	Female	30	Single	Weaver	do	do	do	do	do	do	do	do	do	do	6 brothers	Do.
26	Rocela Azucena	do	15	do	Spinner	do	do	do	do	do	do	do	do	do	do	do	Do.
27	Marcelino Arguellos	Male	40	Married	Laborer	do	do	do	do	do	do	do	do	do	None	4 brothers and 5 children.	Do.
28	Antolin Arguellos	do	35	do	Carpenter	do	do	do	do	do	do	do	do	do	do	2 brothers and 3 children.	Do.
29	Eduvigis Malabanan	Female	12	Child	At home	do	Tanauan, Batangas	White	Reddish	do	Blue	Reddish	do	Feeble	do	3 brothers	Feet and hands too feeble to walk or to support anything; can scarcely see in the daytime, but can see at night.
30	Jorge Fumaya	Male	2.5	do	None	do	Batangas, Batangas	do	do	Yellow	Yellow	Black	do	Normal	do	do	
31	Cresenciano Gonzales	do	19	Single	Student	do	do	do	do	do	do	do	do	Intelligent	do	1 brother	
32	Anselmo de Castro	do	58	do	Laborer	do	Ibaan, Batangas	do	do	White	Reddish	do	do	Feeble	do	5 brothers	
33	Lucena Caringal	Female	26	Married	Cook	do	do	do	Black	do	do	do	do	Regular	Grandmother	2 brothers	
34	Andrea Caringal	do	3	Child	None	do	do	do	Reddish	do	do	do	do	Feeble	do	None	
35	Ismenia Mendoza	do	35	Married	Weaver	do	Lipa, Batangas	White, slightly florid	do	do	do	Natural	do	Regular	do	1 child	

[a] Data collected and tabulated by Villafranca. Mr. Vanden Broeck, ex-provincial treasurer of Union, refers to having seen in Union Province various cases of albinism, with very red hair and white skin; Captain Read, of the Constabulary, refers also to having seen various cases of complete albinos in some villages of Cebu Province; a traveler of Bohol states that he has seen entire families of albinos in the mountains of Samar Province; Dr. Lopez y Lubelza mentions various albinos in Macabebe, Pampanga, and in Agusan, a mountainous province; Sr. Mendoza, third member of the provincial board of Bohol, also states that he has seen entire families of albinos in Siquijor, Oriental Negros; Dr. Molloy has seen two albinos in Baliuag, Bulacan, and one case of an Igorot albino in Bontoc subprovince; Dr. S. Reyes refers to having seen an albino in Taytay, Rizal, and the president of the municipal board of health of Pasig, Rizal, also states that he saw an albino with the hair, as well as all the skin, very white.

[b] Months.

TABLE II.—*Albinism among Filipinos in the Philippine Islands*—Continued.

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														
36	Domingo de Silva	Male	15	Single	Farmer	Total	Lipa, Batangas	White	Reddish	White	Reddish	Natural	Normal	Regular	2 brothers	None	
37	Maria de Silva	Female	4	Child	Student	do	do	do	do	do	do	do	do	do	do	do	
38	Primo de Silva	Male	*8	do	None	do	do	do	do	do	do	do		do	do	do	
39	Petra Hernandez	Female	16	Single	Weaver	do	do	do	do	do	do	Normal	Normal	do	None	do	
40	Feliciana Cuevas	do	35	do	do	do	do	Slightly florid	do	do	do	do	do	do	do	6 brothers	
41	Felix Reyes	Male	10	do	Student	do	do	White	Very red	do	do	do	do	do	Grandfather, great-grandfather, and 3 brothers.	2 brothers	Myopia.
42	Dominga Reyes	Female	4	do	do	do	do	Florid	do	do	do	do	Very precocious	Precocious	do	do	
43	Isabel Magsim	do	29	do	Weaver	do	Taal, Batangas	do	Reddish	Blue	Blue	Light blue	do	Normal	None	None	
44	Remigio Magsim	Male	56	Widower	Laborer	do	do	do	do	do	do	do	do	do	do	do	
45	Maria Magsim	Female	24	Single	Weaver	do	do	do	do	do	do	do	do	do	do	do	
46	Julian Magsim	Male	23	Married	Laborer	do	do	do	do	do	do	do	do	do	do	do	
47	Feliza Casañas	Female	39	do	At home	do	Batangas, Batangas	do	do	Yellow	Yellowish	Black	do	Intelligent	do	1 sister	
48	Marcial Ochoa	Male	15	Single	Student	do	do	do	do	do	do	do	do	do	do	1 brother	
49	Pedro Lizo	do	70	Married	Laborer	Partial	Alburquerque, Bohol	Red	do	Transparent gray.	Transparent gray.	Light blue	do	Normal	do	6 brothers and 9 children.	Nystagmus, strabismus very apparent in both eyes, and lack of pigment; no myopia.
50	Estefania Andoy	Female	50	do	Weaver	do	do	White	do	Blue	Blue	Blue	Feeble	Feeble	Mother and relatives	5 brothers	Ruddy skin in youth, darkening with advancing age.
51	Librada Magdales	do	14	Single	Student	do	Dimiao, Bohol	Slightly pale	do	do	do	do	Normal	Precocious	None	2 brothers	Slight strabismus, slight photophobia. No myopia.
52	Anastasia Dortia	do	30	Married	Weaver	do	do	Florid	do	do	do	do	Feeble	Normal	Father and brothers		Eyebrows and lashes slightly reddish.
53	Ruperto Sale	Male	1	Child	None	do	do	Rosy	do	Transparent gray.	Transparent gray.	do	do	do	Mother and maternal grandparents.		Head large; feet short; eyebrows and lashes slightly reddish.
54	Maximina Daitia	Female	8	do	Student	do	do	do	do	Blue	Blue	do	do	do	Brothers, grandfather, and cousins.		Eyebrows and lashes normal.
55	Victoriana Daitia	do	10	do	do	do	do	do	do	Transparent gray.	Transparent gray.	do	do	do	do		Eyebrows and lashes slightly reddish.
56	Constancia Daitia	do	5	do	do	do	do	do	do	do	do	do	Normal	do	do		Do.
57	Leoncio Daitia	Male	4	do	None	do	do	do	do	Blue	Blue	do	do	do	do		Visual disturbances; eyebrows and lashes black.
58	Angel Daitia	do	*11	do	do	do	do	White	Slightly red	do	do	do	do	do	do		Eyebrows and lashes slightly reddish.
59	Aquilino Laguitas	do	6	do	Student	do	do	White and rosy	Reddish	do	do	do	do	do	Cousin	1 brother	Do.
60	Felicidad Laguitas	Female	3	do	None	do	do	Slightly pale	do	Transparent gray.	Transparent gray.	do	do	do	Great grandmother		
61	Gregorio Cahilog	Male	14	Single	Student	do	Valencia, Bohol	White, slightly ruddy	Slightly reddish	Normal	Normal	Normal	Feeble	do	Great grandmother (maternal).	5 brothers	Eyebrows and lashes black.
62	Rufina Cagaling	Female	9	Child	do	do	do	Rosy	Reddish	do	do	do	Normal	do	Paternal grandparents and brothers.	8 brothers	Eyebrows reddish and lashes black.
63	Casimira Loma	do	12	do	do	do	Panglao, Bohol	Normal	Auburn	Transparent gray.	Transparent gray.	Black	do	Feeble	Aunt and brothers		Timid and nervous. Light reddish coloration around the cornea and reddish streaks on whole surface. No visual disturbances.
64	Junna Balais	do	9	do	do	do	Dimiao, Bohol	Pale	Dark red	Normal	Normal	Blue	do	Normal	Mother, uncle, and great grandmother.	12 brothers	Eyebrows somewhat reddish and lashes black.
65	Procopio Namoco	Male	2	do	None	do	do	do	Reddish	do	do	Somewhat blue.	do	do	Aunt and great grandmother.		Eyebrows reddish and lashes black.
66	Librado Namuag	do	3	do	do	do	do	do	Very red	Transparent gray.	Transparent gray.	Blue	Strong	do	Mother		Eyebrows and lashes somewhat reddish.
67	Marcela Jamito	Female	25	Married	Weaver	do	do	do	do	Pink	Pink	do	do	do	Father and brothers		Eyebrows and lashes black.
68	Marcela Galorio	do	15	Single	do	do	Jagna, Bohol	White, somewhat rosy.	Black	Pinkish gray	Pinkish gray	do	do	Considerably precocious.	Mother, grandfather, aunt, and cousin.		Body short; also feet and hards short.
69	Eugenio de la Sierra	Male	50	Married	Fisherman	do	Cortes, Bohol	Ruddy	do	Normal	Normal	Somewhat reddish.	Normal	Normal	Two daughters		Reddish hair in youth. Paternal grandmother was Spanish.

* Months.

TABLE II.—*Albinism among Filipinos in the Philippine Islands*—Continued.

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
									Hair.	Iris.						
										Color.	Radiation.	Pupil.				
			Yrs.													
70	Albina de la Sierra	Female	5	Child	Student	Partial	Cortes, Bohol	Rosy	Reddish	Normal	Normal	Black	Normal	Normal	Father and sister		Eyebrows and lashes slightly reddish.
71	Leoncia Pabalán	do	14	Single	do	do	León, Bohol	White	Slightly reddish	Blue	Blue	Blue	Strong	do	Parents	4 brothers	Eyebrows and lashes dark.
72	Paterno Pabalán	Male	3	Child	None	do	do	Pale	do	do	do	Black	Feeble	Feeble	do	do	Eyebrows and lashes slightly reddish.
73	Marta Oreullo	Female	30	Married	Weaver	do	do	Ruddy	Light	do	do	Normal	Strong	Normal	Father		Eyebrows and lashes black.
74	Gregoria Salomón	do	21	do	do	do	do	Pale	Slightly reddish	do	do	Blue	do	do	Father, aunt, and paternal grandfather.		Eyebrows and lashes slightly reddish; very reddish when young.
75	Nemesio Libadizos	Male	3	Child	None	do	do	do	do	do	do	Normal	Feeble	do	Father	None	Eyebrows and lashes reddish.
76	Januaria Echavia	Female	5	do	Student	do	Maribojoc, Bohol	Somewhat sunburnt	do	do	do	do	do	do	None		Eyebrows and lashes black.
77	Deogracias Baraso	Male	10	do	do	do	do	Pale	do	do	do	do	Normal	do	do	3 brothers	Do.
78	Simeón Valgas	do	10	do	do	do	do	Brownish	do	do	do	do	do	do	do	6 brothers	Do.
79	Gregorio Pastor	do	8	do	do	do	do	do	do	do	do	do	do	do	do	7 brothers	Do.
80	Severino Monera	do	9	do	do	do	do	do	do	do	do	do	do	do	Brother	3 brothers	Do.
81	Pasiano Descallar	do	5	do	do	do	do	White	do	do	do	do	do	do	do	2 brothers	Eyebrows and lashes slightly reddish.
82	Jose Descallar	do	8	do	do	do	do	Pale	do	do	do	do	do	do	do	do	Eyebrows and lashes black.
83	Celodonia Jumamil	Female	50	Widow	Weaver	do	do	White	Reddish	do	do	do	Feeble	do	Mother and maternal grandfather.	1 child	Eyebrows and lashes reddish.
84	Petronila Arana	do	5	Child	Student	do	do	Pale	do	do	do	do	Normal	do	None		Eyebrows and lashes black.
85	Felisa Canlas	do	5	do	do	do	do	do	do	do	do	do	Feeble	do	Maternal grandparents	2 sisters	Do.
86	Enrica Pastor	do	2	do	None	do	León, Bohol	White and fine	do	do	do	do	do	do	Mother		Somewhat strabismic and with photophobia. Eyebrows and lashes black.
87	Cecilia Valderosa	do	30	Married	Weaver	do	do	do	do	do	do	do	Normal	do	None		Eyebrows and lashes black.
88	Ana Ceballos	do	5	Child	Student		do										
89	Anatalia Candog	do	7	do	do	Partial	Calape, Bohol	White	Reddish	Blue	Blue	Normal	Normal	Normal	Father and brothers	4 brothers	Eyebrows and lashes slightly reddish.
90	Constancia Buston	do	3	do	None	do	Sierra-Bullones, Bohol	Slightly florid	do	do	do	do	do	do	Great grandfather		Eyebrows and lashes reddish; slight myopia.
91	Tomasa Calimbayan	do	4	do	do	do	Tagbilaran, Bohol	White	do	do	do	Blue	Strong	do	None	2 brothers	Eyebrows and lashes slightly reddish.
92	Maxima Enriquez	do	3	do	do	do	do	Rosy	do	do	do	do	do	do	Great grandfather	3 brothers	Eyebrows reddish and lashes black.
93	Alberta Bumaat	do	12	do	Student	do	do	Pale	Slightly reddish	do	do	do	Normal	do	Mother	2 brothers	Eyebrows slightly reddish; lashes black.
94	Dionisia Calizar	do	3	do	None	do	Baclayón, Bohol	Discolored white	Reddish	Light blue	Light blue	do	do	do	Mother and brothers	1 brother	
95	Petrona Calizar	do	8	do	Student	do	do	Rosy	Fine, golden	Blue	Blue	Normal	do	do	do	do	
96	Julia Castrodes	do	18	Single	do	do	Guindulman, Bohol	White	Reddish	Pink	Pink	Blue	do	do	Maternal grandfather and 1 brother.	6 brothers	
97	Ruperto Castrodes	Male	7	Child	do	do	do	Dark red	Dark red	Dark red	do	do	do	do	do	do	
98	Marta Aporinguis	Female	52	Married	Weaver	do	do	Bright red	do	do	do	do	do	do	Father		
99	Victoria Plaza	do	8	Child	Student	do	do	do	do	do	do	do	do	do	Grandfather		
100	Francisco Olayvar	Male	3	do	None	do	do	Rosy	do	Gray	Gray	Red	do	do	Aunt	4 brothers	
101	Fructuosa Racho	Female	14	Single	Student	do	Balilijan, Bohol	do	do	do	do	Gray	do	do	Mother, paternal grandmother, and 4 brothers.	5 brothers	
102	Engracia Racho	do	5	Child	do		do	White	do	do	do	do	do	do	2 brothers		
103	María Libres	do	9	do	do		Anda, Bohol	do	do	Blue	Reddish	Blue	do	do	Maternal grandmother	1 brother	
104	Celestino Bernido	Male	22	Single	Laborer		do	do	do	Pink	Pink	do	do	do	Uncles	5 brothers	
105	Lorenzo Bernido	do	13	Child	Student		do	Dark red	do	do	Gray	do	do	do	Uncles and brothers		
106	Francisca Curan	Female	45	Married	Weaver		Mabini, Bohol	do	do	do	Pink	do	do	do	Father		
107	José Ramirez	Male	6	Child	Student	Partial	Balilijan, Bohol	Dark brown	do	Gray	Gray	do	do	do	1 brother	6 brothers	
108	Albina Cabrera	Female	5	do	do		do	Rosy	do	do	do	do	do	do	None	do	Paternal grandmother is Spanish mestiza.
109	Victoria Torregosa	do	16	Widow	Weaver		Getafe, Bohol	White	do	Reddish	Reddish	do	do	do			
110	Faustino Halasan	Male					Dimiao, Bohol										
111	Jacoba Madronero	Female					do										
112	Filomeno Soria	Male					Pangiao, Bohol										
113	Casimira Soriea	Female					do										
114	Concepción Gimenes	do					Getafe, Bohol										
115	Eusebia Torrefiel	do	16	Married	Weaver	Partial	Mabini, Bohol	Light red	Reddish	Dark red	Gray	Reddish	Normal	Normal	Father	None	
116	José Gela	Male	18	Single	Laborer		Guimbal, Iloilo	White	do	Gray	do	Normal	do	do	3 brothers		Grandfather was albino.
117	Cruz Servidad	do	11	Child	Student		San Joaquín, Iloilo	do	do	do	do	do	do	do	1 brother		Grandfather was albino with history of consanguinity.

TABLE II.—*Albinism among Filipinos in the Philippine Islands*—Continued.

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														
118	Sabina Sanogal	Female	28	Single	Weaver		San Joaquin, Iloilo	White	Reddish	Gray	Gray	Normal	Normal	Normal	2 brothers		Albinism of the family originating from ancestors.
119	Felipe Satorneo	Male	48	Married	Laborer	do	do	do	do	do	do	do	do	do	1 brother		Uncle was albino.
120	Damiana Faimuren	Female	15	Single	Weaver	do	do	do	do	do	do	do	do	do	do		
121	Ma. Magdalena Sardá	do	20	do	do	do	do	do	do	do	do	do	do	do	Albino ancestors		
122	Jesus Sarmonía	Male	6	Child	Student	do	do	do	do	do	do	do	do	do	Paternal grandfather		
123	Caridad Montiosa	Female	16	Single	Weaver		Miagao, Iloilo	do	do	do	do	do	do	do	Albino ancestors		
124	Matilde Nente	do	24	do	do	do	do	do	do	do		do	Good	Sound	1 brother		Albinism of the family originating from ancestors.
125	Vicente Nente	Male	20	do	Laborer	do	do	do	do	do	do	do	do	do	do		Do.
126	Nicasio Nente	do	11	Child	Student	do	do	do	do	do	do	do	do	do	None		Do.
127	Paula Nerva	Female	40	Single	Weaver	do	do	do	do	do	do	do	do	do			
128	Apolonio de la Cruz	Male	40		Laborer	Partial	Jaro, Iloilo	do	do	do	do	do	Normal	Normal	None		
129	Agustin Grajo	do	35		Farmer	do	do	do	do	do	do	do		do	do		
130	Anselmo Laysón	do	65		do	do	do	do	do	do	do	do					
131	Escolastica Octaviana	Female	4	Child	None		Santa Barbara, Iloilo	do	do	do	do	do	Normal				
132	María Tandicada	do	12	do	Student	Total	Pototan, Iloilo	do	Chestnut	Blue	Light blue	Bright blue	Normal and nervous.	Normal	None		
133	Estrella Tandicada	do	5	do	None	do	do	do	do	do	do	do	do	do	do		
134	Probo Tandicada	Male	3	do	do	do	do	do	do	do	do	do	do	do	do		
135	Paula Tamayo	Female	50	Widow	Weaver	do	do	do	do	do	do	do	do	do	Sister		Grandfather was albino.
136	Salvación Gela	do	20	Single	do		Guimbal, Iloilo	do	Reddish	Gray	Normal		Normal	do	do		Do.
137	Silveria Gela	do	1	Child	None		do	do	do	do	do	do	do	do	do		Do.
138	Paula Gela	do	3	do	do		do	do	do	do	do	do	do	do	do		Do.
139	Vicenta Guerrero	do	10	do	Student		do	do	do	do	do	do	do	do	1 brother		
140	Guadalupe Sapulan	do	6	do	do		San Joaquin, Iloilo	do	do	do	do	do	do	do	do		
141	Anunciación Sapulan	do	4	do	None		do	do	do	do	do	do	do	do	None		
142	Carmen Morada	do	16	Single	Weaver		Miagao, Iloilo	do	do	do	do	do	do	do	do		
143	Patricia Noevla	do	5	Child	None		do	do	do	do	do	do	do	do	Grandmother and sister	8 brothers and 5 children.	
144	Juan Balizacan	Male		Married	Laborer	Partial	Solsona, Ilocos Norte	Rosy	do	Light gray	Gray	Reddish	do	do			
145	Alipio Cajigal	do	26	Single	do	Total	Badoc, Ilocos Norte	White	White	Dark blue	Blue	Dark	do	Feeble and limited.	Great grandfather	3 brothers	
146	Calixta Cajigal	Female	34	do	Spinner	do	do	do	do	do	do	do	do	do	do	do	
147	Florencia Cajigal	do	24	do	do	do	do	do	do	do	do	do	do	do	None	None	
148	Calixto Raña	Male	27	do	Laborer	do	do	do	do	do	do	do	do	do	do	2 brothers	
149	Andres Mercado	do	60	do	do	do	do	do	do	do	do	do	do	do	do	do	
150	Castor Mercado	do	17	do	do	do	do	do	do	do	do	do	do	do	do	do	
151	Juan Mercado	do	37	do	do	do	do	do	do	Dark brown	do	do	do	do	do	do	
152	Veneranda Mercado	Female	2	Child	None	do	do	do	do	do	do	do	do	do	do	None	
153	Miguel Lacuesta	Male	21	Single	Laborer	do	do	do	do	do	do	do	do	do	Great grandfather	do	
154	Luisa Lafrades	Female	7	Child	Student	do	do	do	do	do	do	do	do	do	do	do	
155	Rosa Rañases	do	51	Single	Spinner	do	do	do	do	do	do	do	do	do	None	do	
156	Guillerma Calayeay	do	55	do	do	do	do	do	do	do	do	do	do	do	Great grandfather	do	
157	Eduvigis Rañeses	do	38	do	do	do	do	do	do	do	do	do	do	do	None	do	
158	Gregoria Manical	do	49	Married	do	Partial	do	do	do	do	do	do	do	do	Great grandfather	do	
159	Timoteo Rañeses	Male	52	Single	Laborer	Total	do	do	do	do	do	do	do	do	None	do	
160	Candida Palfor	Female	20	do	Spinner	do	do	do	do	do	do	do					
161	Mariano Agudacaldo	Male	62	Married		do	Pasuquin, Ilocos Norte										
162	Ambrocio Daniel	do	15	Single		Partial	do										
163	Ana Caraang	Female	58	Married	House servant	do	San Nicolas, Ilocos Norte.	Rosy	Reddish and gray	Gray	Gray	Dark blue	Character good and temperate; constitution feeble.	Sound	None	2 children	

TABLE II.—*Albinism among Filipinos in the Philippine Islands*—Continued.

No.	Name.	Sex.	Age.	Condition.	Occupation.	Degree of albinism.	Residence.	Skin.	Hair.	Eyes.			Character and constitution.	Mentality.	Other members of the family albinos.	Other members of the family not albinos.	Remarks.
										Iris.		Pupil.					
										Color.	Radiation.						
			Yrs.														
164	Maria Naval	Female	31	Married	House servant	Total	Cabugao, Ilocos Sur	White, coarse	White	Pink	Pink	White	Happy and robust.	Good	1 brother	2 brothers	
165	Valentin Sevilleja	Male	29	do	Laborer	do	do	do	Black	do	Black	Deep red	Regular and delicate.	do	None	None	
166	Clara Naval	do	30	do	do	do	do	do	do	do	Pink	do	Regular	do	1 brother	2 brothers	
167	Severina Pascua	Female	45	Single	Weaver	Partial	Candon, Ilocos Sur	White	Reddish	Blue	Normal	Blue	Good	Feeble			Father, died aged 52, mother 60, not albinos.
168	Fulgencia Pascua	do	42	do	do	do	do	do	do	do	do	do	do	do			Do.
169	Catalina Pascua	do	32	do	do	do	do	do	do	do	do	do	do	do			Do.
170	Santiago Pascua	Male	35	do	Laborer	do	do	do	do	do	do	do	do	do			Do.
171	Gregoria Tadao	Female	68	Married	Spinner	do	Sinait, Ilocos Sur	do	do	Between gray and natural.	do	Normal	Normal	Normal	None	2 brothers	
172	Pablo Tadao	Male	65	Widower	Carpenter	do	do	do	do	do	do	do	do	do	do	do	
173	Federico Inay	do	3	Child	None	do	do	do	do	do	do	do	do	do	do	None	
174	Bernardino Andeja	do	5	do	do	do	do	do	do	do	do	do	do	do	do	do	
175	Petra Ebañes	Female	27	Widow	Weaver	do	do	do	Black	do	do	do	do	Feeble	Grandfather and sister	3 brothers	Has a whitish spot exterior of eyelid.
176	Andres Farnon	Male	15	Single	Fisherman	do	Lapog, Ilocos Sur	do	Reddish	Gray	Gray	Red	do	Feeble	Grandfather and sister	3 brothers	Feet wide, hands short and thick, ears long and wide.
177	Sotero Cabasug	do	11	Child	Student	Total	Cabugao, Ilocos Sur	White, coarse	Red	Pink	Pink	Bright red	Happy and regular.	Good	2 brothers	1 brother	
178	Alberto Cabasug	do	6	do	do	do	do	do	do	do	do	do	do	do	do	do	
179	Graciana Serrano	Female	30	Single	House servant	do	do	do	White	do	do	do	Happy and robust.	do	1 sister	1 sister	
180	Gregoria Serrano	do	25	do	do	do	do	do	do	do	do	do	do	do	do	do	
181	Gertrudes Cabasug	do	4	Child	None	do	do	do	Reddish	do	do	do	Regular	do	2 sisters	2 sisters	
182	Consuelo Arévalo	do	5	do	do		Quiapo, Manila	White spots	Flaxen	Light pink	Light pink	Red	Feeble	Precocious	1 cousin, mother's side		Somewhat myopic and appears photophobic.
183	Joaquín Villanueva	Male	12	do	Student	Total	Victorias, Negros Occidental.	White	Light red	Light gray	Light gray		Strong	According to age		2 brothers	Photophobia. The father of Joaquín Villanueva is nephew of the father of Victorino de León. The father of Victorino de León is second cousin of his mother. Albinism of these congenital.
184	Victorino de León	do	42	Single	Merchant	do	Silay, Negros Occidental.	do	Dark red	Somewhat gray.	do	Dark gray	do	Normal	1 sister	do	Photophobia.
185	Carmen de León	Female	40	do	do	do	do	do	Dark	do	do	do	do	do	do	do	Do.
186	Elias Panega	Male	42	Widower	do	do	Himigarán, Negros Occidental.	do	Red	Reddish gray	do	Reddish	do	do	3 sisters	3 children	Do.
187	Guillerma Panega	Female	52	Married	Weaver	do	do	do	Light red	Somewhat gray.	do	Somewhat reddish.	According to age	do	None	1 brother	Do.
188	Antonino Cidalanga	Male	8	Child	Student	do	do	do	do	Light gray	do	Reddish	do	do	5 brothers and relatives	7 brothers	Do.
189	Tomás Norbe	do	12	do	do	do	do	do	Dark red	Somewhat pink.	do	Dark red	Strong	do	5 brothers (dead)	2 children	Do.
190	Eriberto Beloya	do	41	Widower	Municipal police	do	Murcia, Negros Occidental.	do									
191	Virginia Zaratan	Female	8	Child	Student	Partial	Salasa, Pangasinan	Rosy	Light red	Gray	Gray	Black					
192	Cabina Zaratan	do	2	do	None	do	do	Light flaxen	Light yellow	do	do	do					
193	Dionisia Costales	do	8	do	Student	Total	San Fabian, Pangasinan.	Rosy	Yellowish	do	do	Reddish					
194	Macario Arellano	Male		Single	do	do	San Manuel, Pangasinan.	Light red	White slightly reddish.	do	do	Gray			Maternal grandfather		
195	Antonino Salamanca	do		Married	Laborer	do	do	do	Black slightly reddish.	do	do	Black			Grandfather and 3 brothers.		
196	Faustina Almonte	Female		do		Partial	Manaoag, Pangasinan	Slightly reddish	Yellowish red	do	do	do			None		
197	Emiterio Menor	Male	6	Child	Student	Total	Santa Cruz, Zambales	White	Reddish	do	Somewhat brown	Red	Normal	Normal	1 paternal aunt		
198	Isabel Daria	Female	22	Single	Merchant	do	Iba, Zambales	Pale, ruddy in the face.	Reddish yellow	do	Gray	Black	do	Better than other girls.	Father and 1 brother	4 sisters	Seems to have been much whiter than at present.

THE LIFE HISTORY OF ŒSOPHAGOSTOMUM APIOSTOMUM: I. DEVELOPMENT OUTSIDE OF THE HOST

By ERNEST LINWOOD WALKER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Four plates

In 1905 Brumpt reported a case of œsophagostomiasis in a Negro from the Omo River near Lake Randolph in East Africa. The worm in this case was described by Railliet and Henry (1905) under the name *Œsophagostomum brumpti.*

Thomas, in 1910, found a fatal case in a native of Manaos, Brazil, and described the pathological anatomy and histology of the case very completely. The worm in this case was likewise referred to Railliet and Henry for identification. These authors described the parasite under the name *Œsophagostomum stephanostomum* var. *thomasi* Railliet and Henry, 1910.

Leiper, of the London School of Tropical Medicine, in 1911, found among the hookworms collected by Doctor Foy, of the West African Medical Staff, 6 œsophagostoma, which had been passed in the stools of a native at Ibi, North Nigeria. Leiper identified the worms in this case as *Œsophagostomum apiostomum* Willach, 1891, a species common in the intestine of apes. Furthermore, this author is of the opinion that *O. brumpti* Railliet and Henry, 1905, is identical with *O. apiostomum* Willach.

These are the only cases of the infection of man with this worm that have so far been reported, but Weinberg (1908) believes that the few cases in man so far reported in Africa are due to the fact that necropsies on Negroes are rare in the African colonies and that attention has been directed especially to parasites of the blood.

Beside man and apes, cattle, sheep, goats, pig, and *Dasypus* are subject to œsophagostomiasis, but in these latter animals the infection is due to other species of *Œsophagostomum.*

Œsophagostomiasis is characterized by hæmorrhagic cysts or tumors in the submucosa or muscularis of the large intestine—rarely of the small intestine—which project usually both inside and outside of the gut, and which contain the immature adult œsophagostomum. At maturity the cyst ruptures and the adult

worm or of *Strongyloides*, the latter of which are frequently numerous in the same culture of monkey's fæces. The newly hatched œsophagostomum larva is characterized: first, by an extremely long filiform tail and, secondly, by the zigzag course of the intestine which is plainly visible in the living worm. These two characteristics are well represented in Plate I, fig. 3, and Plate II, fig. 2.

The small rhabditiform larva grows rapidly, and under favorable conditions of culture and temperature attains its maturity in from three to four days. In the process of growth it molts twice. At the last molt the old skin is not shed, but remains as a sheath inclosing the larva. Within the sheath the larva contracts somewhat in breadth and more in length, so that it is separated from the old larval skin by a considerable space. The larva no longer possesses the long filiform tail, which was present up to the last molt, as is seen from the inclosing larval skin (Plate III, fig. 1). The character of the œsophagus has also changed during the last molt from the rhabditiform to the strongyliform (compare Plate II, fig. 1, with Plate IV, fig. 1). This larva inclosed in the skin of the last molt remains alive and active, but undergoes no further development in the culture. It is the mature larva ready to infect a new host. It differs from the mature larva of the hookworms and strongyloides in size and shape, and especialy in the long filiform tail of the old larval skin inclosing the worm. The mature larva is about 0.9 millimeter long and 0.03 millimeter thick.

The larval development of *Œsophagostomum apiostomum*, therefore, is strikingly similar and wholly comparable with the development of *Ankylostoma duodenale*, which is perhaps to be expected from the somewhat near relationship of the two worms. From this similarity in the development of the larva, one would expect by analogy that the method of entrance of the larva into the body of the host would also be similar. It has been demonstrated by Looss (1911), and substantiated by other investigators, that infection with ankylostoma larvæ may take place not only by ingestion, but also by passage through the moistened skin. This is supposed to take place especially in case of persons going about barefooted. The larvæ enter the follicles and attain the blood vessels, by which they are carried to the lungs. Here they leave the blood and enter the air vesicles and then travel by the way of the bronchi, trachea, œsophagus, and stomach to the intestine. The method by which the larva of œsophagostomum enters the body of its host and attains its position in the tissues of the large intestine is now being experimentally investigated.

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ILLUSTRATIONS

Plate I, fig. 3, and Plate IV, figs. 1 and 2, are reproductions of camera-lucida drawings by Teodosio S. Espinosa, the remaining figures are reproductions of photomicrographs by Charles Martin. Plate I, fig. 2, and Plate II, fig. 2, are from stained preparations, the others from unstained preparations killed in 70 per cent alcohol and mounted in glycerine.

PLATE I

FIG. 1. Ovum of *Œsophagostomum apiostomum*. × 350.
2. Young rhabditiform larva. × 84.
3. Young rhabditiform larva at higher magnification, showing the filiform tail, intestinal tract, and *anlarge* of reproductive organs. × 390.

PLATE II

FIG. 1. Cephalic end of young rhabditiform larva, showing shape of œsophagus. × 350.
2. Body of young rhabditiform larva, showing undulating course of intestinal tract. × 350.
3. Young rhabditiform larva, showing posterior end of intestinal tract and anus. × 350.

PLATE III

FIG. 1. Mature larva inclosed in skin of last molt. × 84.
2. Cephalic end of mature larva inclosed in skin of last molt. × 350.
3. Posterior part of body of mature larva inclosed in skin of last molt × 350.

PLATE IV

FIG. 1. Cephalic end of mature larva, showing strongyliform œsophagus. × 390.
2. Caudal end of mature larva. Note the long filiform tail of old larval skin. × 390.

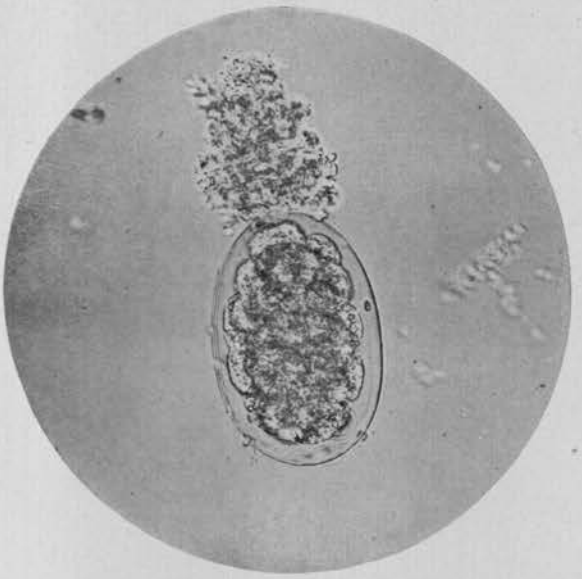

Fig. 1. Ovum. × 350.

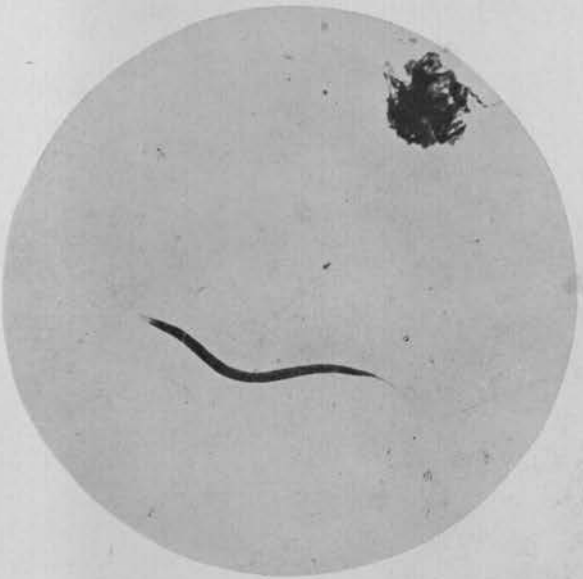

Fig. 2. Young larva. × 84.

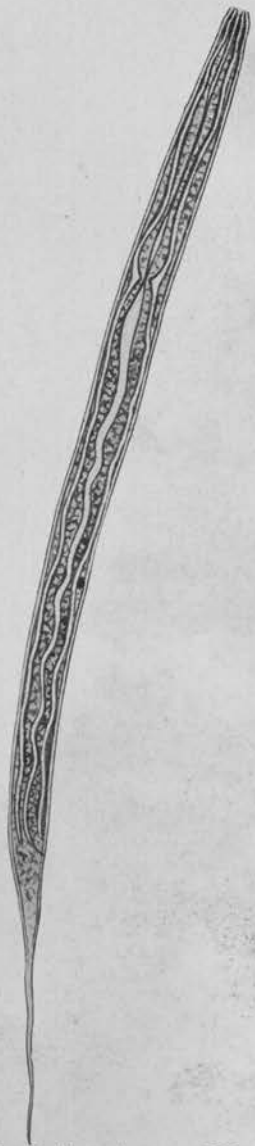

Fig. 3. Young larva × 390.

PLATE I. OVUM AND YOUNG RHABDITIFORM LARVA OF ŒSOPHAGOSTOMUM APIOSTOMUM WILLACH.

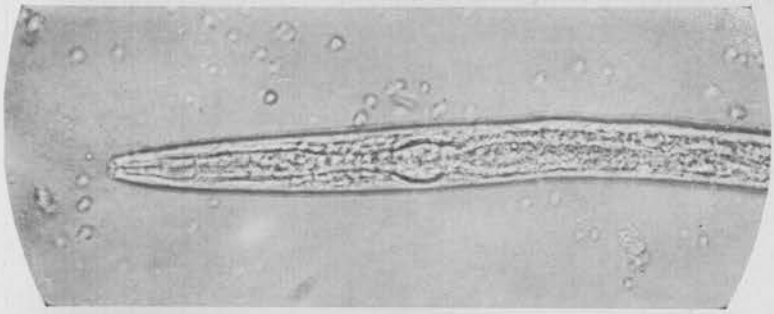

Fig. 1. Cephalic end of young rhabditiform larva, showing shape of œsophagus. × 350.

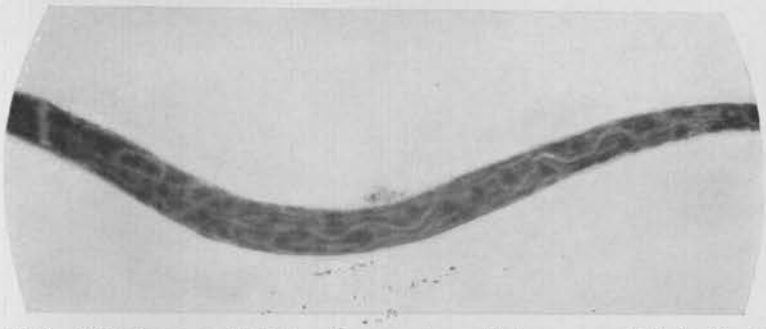

Fig. 2. Body of a young rhabditiform larva, showing undulating course of intestinal tract. × 350.

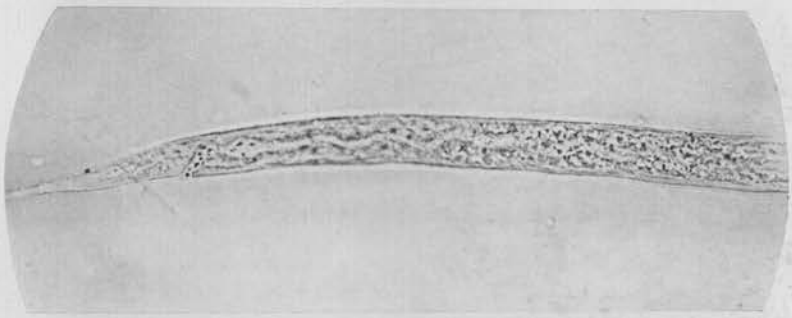

Fig. 3. Young rhabditiform larva, showing posterior end of intestinal tract and anus. × 350.

PLATE II. YOUNG RHABDITIFORM LARVA OF ŒSOPHAGOSTOMUM APIOSTOMUM WILLACH.

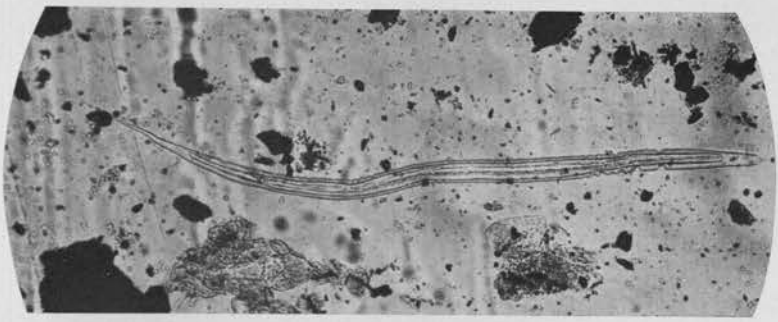

Fig. 1. Mature larva inclosed in skin of last molt. × 84.

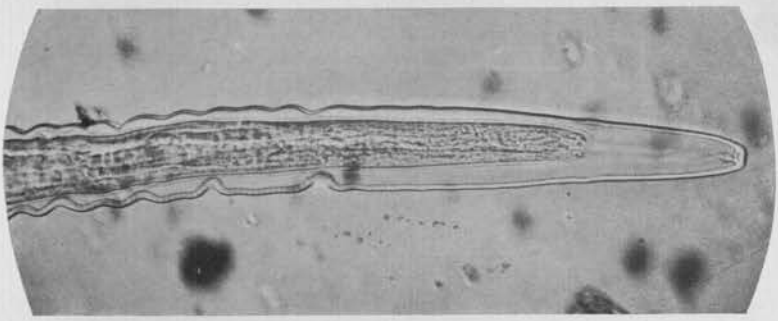

Fig. 2. Cephalic end of mature larva inclosed in skin of last molt. × 350.

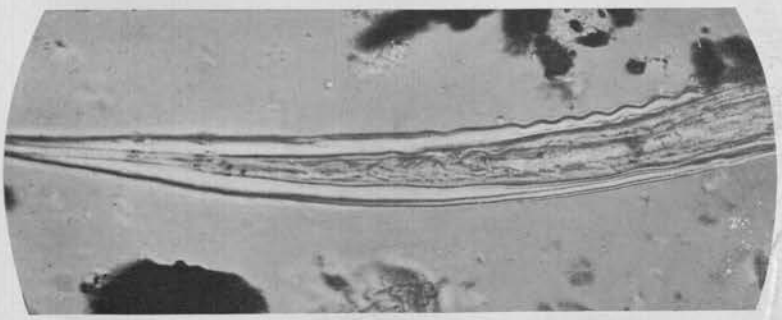

Fig. 3. Posterior part of body of mature larva inclosed in skin of last molt. × 350.

PLATE III. MATURE LARVA OF ŒSOPHAGOSTOMUM APIOSTOMUM WILLACH.

Fig. 1. Cephalic end of mature larva, showing strongyliform œsophagus. × 390.
2. Caudal end of mature larva. Note the long filiform tail of old larval skin. × 390.

PLATE IV. ŒSOPHAGOSTOMUM APIOSTOMUM WILLACH.

DURATION OF THE INFECTIVENESS OF VIRULENT RINDERPEST BLOOD IN THE WATER LEECH, HIRUDO BOYNTONI WHARTON [1]

By WILLIAM HUTCHINS BOYNTON

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This investigation was suggested by the fact that in the campaign against rinderpest in the Philippines particular difficulty is experienced in ridding low swampy districts of the disease. On numerous occasions such localities have been apparently free from rinderpest, but in four or five weeks the disease has reappeared. In most instances the movement of animals was apparently controlled, and it did not seem possible that the disease was introduced from other districts.

Consideration of these apparently spontaneous outbreaks with reference to the localities in which they appeared to be most frequent led me to examine leeches, to determine if they could maintain the virus of rinderpest alive for any length of time. Leeches subsist on blood, and consume large quantities at one feeding. Cattle sick with rinderpest are apt to seek cool places and water holes during the febrile stage of the disease, while carabaos do so normally. This gives the leeches ample opportunity to feed upon them. Persons engaged in field work have repeatedly observed leeches attached to carabaos immediately after they emerged from carabao wallows. These facts indicate that leeches may be a factor in the rinderpest problem.

On examining the literature on leeches, I have found that Bass and Johns cite the statement of Sakharov, Rosenbach, Blumer, Hamburger, and Mitchel(1) that they kept malaria plasmodia alive for several days in leeches that had been allowed to draw the blood of malaria patients.

Laveran and Mesnil (2) state:

> Various trypanosomes which were found by Brumpt in fresh-water fishes, can be divided into several groups according to their mode of evolution in the bodies of leeches (Hemiclipsis).

[1] To be published as Bulletin No. 29, Bureau of Agriculture of the Government of the Philippine Islands.

[2] Archibald R. Ward, Chief.

Elsewhere they state, in discussing a trypanosome disease of horses in Annam, that Vassal(3) found that—

> The blood of leeches which had fed on infected animals was infective, on injection into rats, immediately after the meal of blood, but not four hours later. The trypanosomes are killed off very readily in the stomach of the leech.

Daniels and Alcock state (4):

> Many parasites maintain their virulence for a considerable period in the stomach of leeches, but leeches are not known to act as carriers of disease.

Nencki, Sieber, and Wijnikewitch (5) allowed leeches to feed upon animals infected with rinderpest. Later they examined the blood in these leeches for the presence of the organism regarded by them as the causative agent of rinderpest, but without success.

The leech employed in these experiments is a new species, *Hirudo boyntoni*.[3]

The leeches used in the first of the following experiments were procured from La Carlota, Occidental Negros, a locality which for several years has not been known to be infected with rinderpest. It was thought best to select leeches from such an uninfected region when beginning the study.

On July 13, 1912, ten leeches were allowed to feed on bull 3397, in the second day of febrile temperature of an attack of rinderpest. As soon as the leeches had become engorged, they were placed in water and kept in a cool place. The virulence of the blood in these leeches was tested upon cattle after various intervals, as described in experiments 1 and 2.

Experiment 1.—At 10.00 a. m., July 14, twenty-four hours after the leeches had fed on the sick animal, 2 leeches were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge the blood. The mixture of disgorged blood and salt solution was injected subcutaneously into bull 3396, which was placed in a screened stall. This animal did not contract the disease, but at a later date proved to be susceptible.

Experiment 2.—On July 15, two of the leeches which had fed on July 13, were placed in 50 cubic centimeters of physiological salt solution which caused them to disgorge the blood which they had held for forty-eight hours. This mixture of blood and salt solution was injected subcutaneously into bull 3390. The

[3] Wharton, L. D., *This Journal, Sec. D* (1913), 8, 369.

animal showed a rise in temperature on the morning of July 19, diarrhœa with inappetence on July 22, and died during the forenoon of July 24. The symptoms and lesions gave conclusive evidence that it had contracted rinderpest and died of that disease.

On July 16, 1912, at 11.00 a. m., several leeches were allowed to feed on bull 3402 in the second day of temperature of an attack of rinderpest. As soon as the leeches had become engorged, they were placed in water and kept in a cool place. The infectiveness of the blood contained in these leeches was determined at various intervals by testing upon cattle as shown in experiments 3 to 6.

Experiment 3.—On July 17, 1912, at 11.00 a. m., 2 leeches which had fed on July 16 were placed in 100 cubic centimeters of physiological salt solution which caused them to disgorge the blood which they had held for twenty-four hours. This mixture of blood and salt solution was injected subcutaneously into bull 3405. The animal had a rise in temperature on July 22, developed diarrhœa and inappetence on July 26, and died on July 27. From the symptoms and lesions it was concluded that the animal had contracted rinderpest and died of that disease.

Experiment 4.—On July 21, 1912, several of the leeches which had fed on July 16 died and disgorged blood into the water in which they were being kept. The mixture of water and blood was given as a drench to bull 3404. This animal showed a febrile temperature on July 31, which was ten days after receiving the drench. Diarrhœa with inappetence appeared on August 6 and continued until August 10, after which the animal gradually recovered. The case presented all the symptoms of a severe attack of rinderpest. At a later date the animal received virulent blood and was proved to be immune, thus showing that it had passed through the disease.

Experiment 5.—On July 21, 1912, the dead leeches which were mentioned in experiment 4, and which had fed on an infected animal on July 16, were thoroughly disintegrated in a mortar containing physiological salt solution, and the fluid was injected subcutaneously into bull 3400. This animal showed a high temperature on the evening of July 27, diarrhœa with inappetence on July 31, and died during the forenoon of August 7. From the symptoms and lesions it was concluded that this animal had contracted rinderpest and had died of that disease.

Experiment 6.—On August 2, 1912, two leeches which had fed on July 16, seventeen days previously, were placed in physiolog-

ical salt solution which caused them to disgorge blood. This mixture of blood and salt solution was injected subcutaneously into bull 3473. The animal showed a rise in temperature on August 5, but on August 9 was found positive for surra, which at the time was thought to be the possible cause of the rise in temperature. However, the animal developed diarrhœa and ate but little on August 11, showed inappetence on August 12, and died on August 14. From the symptoms and autopsy findings it was evident that this animal had an attack of rinderpest as well as of surra.

On July 29, 1912, sixteen leeches were allowed to feed on bull 3400, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. These were employed at different periods, shown in experiments 7 to 10, to test upon cattle the infectiveness of the blood that they contained.

Experiment 7.—On August 4, six days after having fed, 2 leeches were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into bull 3477. This animal displayed the first rise in temperature on the evening of August 7, was found to be infected with surra on August 9, and died on August 13. This animal developed nervous symptoms which are characteristic of a somewhat rare type of rinderpest. It also showed a subnormal temperature of 36°.2 C., which is frequently present in rinderpest just prior to death. Autopsy revealed slight lesions of that disease. It was evident that this animal had died from the combined effects of rinderpest and surra.

Experiment 8.—On August 10, 1912, twelve days after feeding, 2 leeches which had fed on July 29 were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The blood and salt solution was injected into bull 3492. The animal showed a rise of temperature on August 14, developed diarrhœa with partial inappetence on August 18, and died on August 19. From the various symptoms and from the autopsy findings, it was concluded that this animal had contracted rinderpest and died of that disease.

Experiment 9.—On August 16, 1912, eight small leeches, twelve days after having fed on July 29, were placed in physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3491. This animal showed no ill effects from the injection, but at a later date was proved to be susceptible to rinderpest.

Experiment 10.—On August 16, 1912, the water in which the leeches of experiment 9 had been kept since July 29—a period of twelve days—was given as a drench to bull 3488. This animal suffered no ill effects from the drench. It was later infected with rinderpest and died, showing that it had been susceptible to the disease at the time it had received the drench.

On August 17, 1912, twenty leeches were allowed to feed upon bull 3492 during the third day of temperature of an attack of rinderpest, and were placed in water in three different containers and kept in a cool place. The infectiveness of the blood contained in these leeches was tested on cattle at various periods in experiments 11 to 14.

Experiment 11.—On August 24, 1912, two leeches which had fed on August 17, seven days previously, were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into animal 3494. This animal showed a rise in temperature on September 3, ten days after receiving the injection. Diarrhœa developed on September 4, and inappetence on September 5. The animal showed a subnormal temperature of 36°.2 C. on the afternoon of September 6, and died that evening. Autopsy showed typical lesions of rinderpest. It was concluded that this animal had contracted a fatal attack of rinderpest.

Experiment 12.—On August 24, 1912, the water in which 8 leeches had been kept since August 17, an interval of seven days, was given by drench to bull 3495. The animal suffered no ill effects from the material. This animal was used in a subsequent experiment in which it was shown to be susceptible.

Experiment 13.—On August 27, 1912, three leeches which had fed on August 17, ten days previously, were placed in 75 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into bull 3488. This animal suffered no ill effects from the injection, and at a later date was proved to be susceptible to rinderpest.

Experiment 14.—On August 27, 1912, the water in which several leeches had been kept since August 17—a period of ten days—was given by drench to bull 3491. This animal suffered no ill effects from the material, but at a later date was proved to be susceptible to rinderpest.

On November 8, 1912, five leeches were allowed to feed on cow 3516, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. The

ineffectiveness of the rinderpest virus in the blood contained in these leeches was tested upon cattle in experiments 15 to 17.

Experiment 15.—On November 18, 1912, one leech, which had fed on November 8, ten days previously, was placed in 50 cubic centimeters of physiological salt solution which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3514. This animal showed an initial rise of temperature on November 23, developed diarrhœa on November 24, and inappetence on November 26. It displayed a subnormal temperature of 36°.7 C. on the morning of November 29, and died during the day. Autopsy showed typical lesions of rinderpest. It was thus proved positively that this animal had suffered a fatal attack of rinderpest.

Experiment 16.—On November 20, 1912, one leech, which had fed on November 8, twelve days previously on an infected animal, was placed in 50 cubic centimeters of physiological salt solution, which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3518. This animal showed the initial rise of temperature on November 26, which continued for several days, gradually subsiding to normal. The animal showed no diarrhœa nor inappetence. Blood was drawn from it and injected into a susceptible bull which developed a severe case of rinderpest and died. This proved that bull 3518 had been infected with a mild type of rinderpest, but had been able to transfer a severe type of the disease to another animal.

Experiment 17.—On November 23, 1912, one leech, which had fed on November 8, fifteen days previously, was placed in 50 cubic centimeters of physiological salt solution, which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3524. This animal showed an initial rise of temperature on November 28, developed diarrhœa with partial inappetence on December 2, and died during the daytime of December 6. The autopsy revealed marked lesions of rinderpest. Therefore, this animal had a fatal attack of rinderpest.

On November 30, 1912, four leeches were allowed to feed on cow 3524, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. The infectiveness of the blood retained in these leeches was tested upon cattle at various intervals as shown in experiments 18 and 19.

Experiment 18.—On December 10, 1912, one leech which had fed November 30, ten days previously, was placed on some green

feed which had been sprinkled with a small amount of sodium chloride. A few minutes after the leech came in contact with the fodder it disgorged a considerable amount of blood. This fodder was fed to bull 3535, and was eaten readily. This feeding had apparently no ill effect upon the animal. At a later date the same animal was proved to be susceptible to rinderpest.

Experiment 19.—On December 18, 1912, two leeches, which had fed on November 30, eighteen days previously, were placed in 100 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3538. This animal showed the initial rise in temperature on December 23, developed diarrhœa and inappetence on December 27, which symptoms continued until January 4, 1913, when the animal died. Autopsy revealed typical lesions of rinderpest. From these observations it was proved that this animal had contracted a fatal attack of rinderpest.

During the early part of January, 1913, I visited the Province of Ambos Camarines to investigate a disease affecting cattle and carabaos. A few animals located in Magarao, a barrio of Nueva Caceres, exhibited symptoms and blood changes which are characteristic of rinderpest, although in a mild form. A large number of leeches were collected in this locality.

On January 15, 1913, four leeches which had been collected in the vicinity were allowed to feed on a young carabao in Magarao which presented some symptoms of rinderpest. It had a rather high temperature, it refused food, the ears drooped, and a somewhat characteristic diarrhœa was present. When the leeches had engorged themselves, they were placed in a bottle partly filled with water, and were brought to the veterinary research laboratory at Alabang, where the blood that they contained was tested upon cattle at various intervals, as shown in experiments 20 and 21.

Experiment 20.—On January 18, 1913, one of the leeches which had fed on the sick carabao in Magarao three days previously was placed in 50 cubic centimeters of physiological salt solution to cause it to disgorge the blood which it contained. This mixture was injected into bull 3543. The animal presented a rise of temperature on January 24, displayed inappetence January 29, diarrhœa on January 30, and showed a bloody diarrhœa on January 31. This animal experienced a rather severe attack of the disease, but recovered.

From the results it was evident that the sick carabao in Magarao had been infected with rinderpest. This decided a matter

concerning which there had been considerable doubt, owing to the absence of well-marked cases.

Experiment 21.—On February 24, 1913, two leeches which had fed on the sick carabao at Magarao, Ambos Camarines, on January 15, forty days previously, were disintegrated in a mortar and the blood was injected into bull 3548. The animal apparently suffered no ill effect from the injection but was proved susceptible to rinderpest at a later date.

On January 22, 1913, twelve leeches were allowed to feed on cow 3535, on the second day of febrile temperature of an attack of rinderpest, and then placed in water in a cool place. The blood contained in these leeches was tested upon cattle in experiments 22 to 27.

Experiment 22.—On February 11, 1913, two leeches which had fed on January 22, twenty days previously, were placed in 100 cubic centimeters of physiological salt solution, which caused them to disgorge blood. This mixture of blood and salt solution was injected into bull 3564. The animal suffered apparently no ill effects from the injection. At a later date it was proved to be susceptible to rinderpest.

Experiment 23.—On February 16, 1913, two leeches which had fed on January 22, twenty-five days previously, were placed in 75 cubic centimeters of physiological salt solution, which caused them to disgorge. This mixture was injected subcutaneously into bull 3566. The animal showed an initial rise of temperature on February 22, developed inappetence on February 27, and diarrhœa on March 1. The inappetence continued to March 4, when the animal again began to eat, but the diarrhœa continued until March 7. This animal gradually recovered. From the symptoms it was concluded that this animal experienced an attack of rinderpest.

Experiment 24.—On February 22, 1913, one leech, which had fed on January 22, thirty-one days previously, was caused to disgorge by means of salt solution. The mixture of blood and salt solution was injected into bull 3549. This injection had apparently no ill effect upon the animal. Its susceptibility to rinderpest was proved later.

Experiment 25.—On February 26, 1913, five leeches, which had fed on cow 3535 on January 22, thirty-five days previously, were allowed to feed on bull 3568. This animal suffered no ill effects from the feeding. It was proved susceptible to rinderpest at a later date.

Experiment 26.—On March 3, 1913, four leeches which had

fed on infected blood on January 22, forty days previously, were disintegrated in a mortar in salt solution. The mixture of blood and salt solution was injected subcutaneously into bull 3547. One of the leeches was dead, 1 was inactive, and 2 were in normal condition at the time they were put in the mortar. The animal apparently suffered no ill effects from the injection. It was proved by a subsequent inoculation to be susceptible to rinderpest.

Experiment 27.—On February 6, 1913, two liters of water were given by drench to bull 3547. Twelve leeches that had fed on infected blood fifteen days previously had been kept in this water. This animal suffered no ill effects from the drench. It was proved susceptible to rinderpest by a subsequent inoculation of virulent blood.

Experiment 28.—On February 8, 1913, three leeches were allowed to feed for fifteen minutes on cow 3546, during the third day of temperature of an attack of rinderpest, and were transferred to water for one hour. They were then placed on susceptible animal 3548, and allowed to become engorged. Cow 3548 suffered no ill effects from the biting of these leeches. She was proved susceptible to rinderpest by a subsequent inoculation of virulent blood.

Experiment 29.—On February 8, 1913, three leeches were allowed to feed for fifteen minutes on cow 3546, on the third day of temperature of an attack of rinderpest, and then transferred, without being placed in water, to susceptible cow 3549. The interval between the two feedings was twenty minutes. Animal 3549 suffered no ill effects from this feeding. It was proved susceptible to rinderpest at a later date by an inoculation of virulent blood.

On March 4, 1913, eleven leeches were allowed to feed for thirty minutes on cow 3564, on the fourth day of febrile temperature of an attack of rinderpest, after which they were tranferred to water in a cool place. The infectiveness of the blood in these leeches was determined by testing upon cattle at various periods subsequently, as shown in experiment 30.

Experiment 30.—On March 10, 1913, the 11 leeches which had fed on cow 3564, on March 4, six days previously, were allowed to feed on cow 3570 until they become engorged. This animal apparently suffered no ill effects from the feeding. She was later proved to be susceptible to rinderpest.

Since the animals used in experiments 6 and 7 had been found to be infected with surra, it was thought best to test leeches for their ability to keep the trypanosome of surra alive in ingested

blood. In the following experiments guinea pigs were used since they are susceptible to surra.

On April 21, 1913, four leeches were fed for fifteen minutes on a guinea pig whose blood was heavily infected with the trypanosomes of surra, after which the leeches were placed in water and kept in a cool place. The infectivity of these leeches with regard to surra was tested in experiments 31 and 32.

Experiment 31.—On April 23, 1913, two days after the leeches had fed, 2 of them were allowed to feed on 2 healthy guinea pigs. These animals were kept under observation for one month, during which time they were not found to be infected.

Experiment 32.—On April 23, 1913, two days after the leeches had fed, 1 leech was placed in a small amount of physiological salt solution and thoroughly disintegrated in a mortar. The ingested bood was examined microscopically for trypanosomes, but none were found. The remaining blood was injected into a guinea pig, which was kept under observation for one month, but did not develop the disease.

Experiment 33.—On April 27, 1913, two leeches were allowed to feed for seven minutes on a guinea pig heavily infected with surra, after which time they were removed, kept out of water, and placed on a healthy guinea pig. They commenced feeding on the healthy guinea pig in seven and in seven and one-half minutes, respectively, after having been taken from the infected animal, and were allowed to feed upon the healthy animal for ten minutes. This guinea pig was kept under observation one month, but remained negative for surra.

Experiment 34.—On April 27, 1913, two leeches were allowed to feed for ten minutes on a guinea pig heavily infected with surra, after which they were placed on a healthy guinea pig. They began feeding on the healthy animal in one hour and two minutes and one hour and five minutes, respectively, after being removed from the infected animal. This guinea pig was kept under observation for one month, but remained negative for surra.

During the early part of May, 1913, I visited the Province of Ilocos Sur to study some mild cases of rinderpest. On May 3, 1913, one leech was allowed to feed on a carabao which had shown no symptoms of rinderpest except ulcers in the mouth. The temperature of this animal had not been observed. It has been my experience to find no ulcers forming in the mouth in the virulent type of the disease until three or four days, and sometimes longer, after the initial rise in temperature. In a mild type of rinderpest, undoubtedly, a longer period than this would

elapse before ulcers would appear. Therefore, it was probable that this animal had nearly recovered and might not have been capable of spreading the disease.

Experiment 35.—On May 8, 1913, the leech, which on May 3 had fed on the carabao showing ulcers in its mouth, was caused to disgorge in physiological salt solution. This mixture of blood and salt solution was injected subcutaneously into bull 3573. The animal showed no ill effects from the injection. It was later proved susceptible to rinderpest.

On May 4, 1913, in Ilocos Sur, one leech was allowed to feed on a carabao which was recovering from an attack of rinderpest. The leech was placed in water and brought to the laboratory. This animal had presented some symptoms of rinderpest; as, for instance, a discharge from its eyes and nose and a mild diarrhœa. At the time the leech was allowed to feed, these symptoms had practically disappeared.

Experiment 36.—On May 8, 1913, the leech, which had been allowed to feed on the recovering animal on May 4, was caused to disgorge by placing it in salt solution. This mixture of blood and salt solution was injected subcutaneously into bull 3574. The animal showed no ill effects from his injection. It was proved to be susceptible to rinderpest at a later date.

CONCLUSIONS

1. From the results obtained in experiment 22 and others it is proved that the large water leech (*Hirudo boyntoni* Wharton) can retain the virus of rinderpest alive in its body for at least twenty-five days and in a virulent condition.

2. From experiment 4 it is shown that water in which leeches have disgorged blood by mechanical stimulation or other means, after holding it for a period of five days, will cause rinderpest when drunk by a susceptible animal.

3. The result obtained in experiment 5 proves that leeches, which have died from mechanical or other cause, after holding virulent blood for five days, are able to transmit the disease when the blood is ingested by a susceptible animal.

4. From experiments 4 and 11 it is shown that an animal may have an incubation period of ten days after being infected with material which has been held by a leech.

5. Experiment 16 shows that an animal may develop a very mild type of the disease when infected from blood that has been held in a leech for several days. Under such conditions an animal experiencing a mild attack may transmit a virulent and fatal

type of rinderpest. This suggests one of the possible causes for mild rinderpest frequently encountered in the field.

6. It was observed that the large leech held the virus alive considerably longer than small leeches.

7. It was noted that when leeches had been out of water for any length of time they disgorged the blood. Carabaos coming from wallows have frequently been observed to be covered with leeches. Carabaos are in the habit of eating grass around the edges of wallows, and there is a possibility of leeches getting out of the water and disgorging blood on the grass. If this were eaten by a susceptible animal within twenty-four hours, there would be a possibility of the animal contracting rinderpest, if the leech previously had fed, within the limits of time the virus is known to remain alive in the ingested blood, on an animal sick with rinderpest.

8. It has been observed that the virus will continue alive much longer in a leech if it be allowed to feed on an animal during the early stages of the disease. The immune bodies formed in the blood serum may have considerable effect on the virus in the later stages of the disease. Since the temperature of an animal suffering from rinderpest is usually highest during the first three or four days after the initial rise, it is possible that animals would seek cool places and water holes during the early stages of the disease rather than during the later period. This condition would give the leech ample opportunity to feed upon animals in the early stage of the disease.

9. Experiments 10, 12, 14, and 27 show that the water in which leeches have been kept is not infective to a susceptible animal, provided the leeches have not been injured in any way that would cause them to disgorge the blood which they contained.

10. The results of experiments 25, 28, 29, and 30 show that leeches cannot transmit the disease to a susceptible animal by feeding on it, after they have fed upon an animal suffering from rinderpest.

11. The results from experiments 31, 32, 33, and 34 indicate that the trypanosome of surra does not remain alive for any length of time in the ingested blood of a leech, and that a leech cannot transmit the disease by biting.

12. From the results obtained in the foregoing experiments, it appears that a leech may be responsible for the appearance of recognizable rinderpest forty days after imbibing virulent blood. Of this period, the leech could hold the blood twenty-five days, and to this may be added an incubation period of ten days, which was observed in one of the preceding experiments.

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THE PHYSICAL AND CHEMICAL PROPERTIES OF THE OLEORESIN OF ASPIDIUM * WITH RESPECT TO THE DETECTION OF ADULTERATIONS

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INTRODUCTION

The ethereal extract of male fern, the so-called "oleoresin of aspidium" of the United States pharmacopœia,[1] since the time of its preparation by Peschier in 1825,[2] has been the most generally used of the numerous tæniafuges to be found in the materia medica; but of late it has been falling into disrepute. This fact is to be attributed directly to the variable results following its administration. That the extract as found upon the market varies to a great extent in physiological action, which is in a measure proportional to its tæniafuge properties, is manifest in the fact that it has caused severe symptoms of poisoning when given in doses of 4 grams;[3] while in other cases, 40 grams[4] have been administered without the appearance of evil symptoms. The lesser activity of the larger dose does not necessarily indicate an adulterated or deteriorated product as it is known that the activity of the extract is dependent upon a number of other factors as well—the locality in which the rhizomes are grown, the time of harvesting, et cetera. Thus, Matzdorff[5] reports a variation of from 0.815 to 4.145 per cent of crude filicin in rhizomes gathered in different parts of Germany and Russia, the highest filicin content being found in the Russian rhizomes. Berenger-Feraud[6] found that extracts prepared from rhizomes gathered in Normandy were much less active than the extracts prepared from rhizomes gathered in the Vosges or in the Jura Mountains.[7]

* The species *Aspidium filix mas* Sw. and *A. spinulosum* Sw. do not properly belong in the genus *Aspidium*, but are now referred by botanists to the genus *Dryopteris*, as *D. filix mas* Schott and *D. spinulosa* O. Kuntze, respectively. As the name *Aspidium* is in general use in pharmacological literature to designate these plants, it is here retained.

[1] The eighth revision of the United States Pharmacopœia directs that the oleoresin of aspidium be prepared by using acetone as the exhausting menstrum, which is rarely if ever done by either American or foreign manufacturers, because of the production of an inferior article.

[2] *Repert. d. pharm.* (1827), **27**, 349.

[3] *Therap. Monatsh.* (1889), **3**, 90–138.

[4] *Deutsch. Arch. f. klin. Med.* (1889), **53**, 348–358.

[5] *Apoth. Zeitg.* (1901), **16**, 578.

[6] *Arch. d. Pharm.* (1886), **224**, 1034, from *Bull. Gen. Therap.*, **110**, 481.

[7] Citation from Reuter, *Pharm. Zeitg.* (1891), **36**, 245–246.

Kobert states that the Russian extract is about ten times as active as the German extract and twenty times as active as that obtained from France. Van der Marck [8] and others have found the extract prepared from the rhizomes gathered in September to be the most active. In addition, it has been observed that individual idiosyncrasies or certain diseases predispose the patient to its toxic effects; [9] likewise, the administration of an oily laxative—castor oil—with the extract is said to increase its toxic action,[10] or the failure to administer any laxative at all may produce intoxication.[11] Taking into due consideration all of the above factors, there is ample evidence both in the literature and the laboratory to show that the adulteration and deterioration of the extract is a large item in connection with the uncertainty of its therapeutic action. The proof for the latter statement will be presented as this paper progresses.

In accordance with our present knowledge, the ethereal extract of male fern owes its tæniafuge properties to the presence of a number of compounds; ketone-like combinations of phloroglucinol, mono-, di-, and trimethyl phloroglucinol with butyric acid and the condensation of 2 (flavaspidic acid, albaspidin, etc.), 3 (filix acid), or 4 (filmaron) such butanones. Poulsson [12] attributes the action to the filix acid alone; Kobert [13] is of the opinion that it is due to an intimate mixture of the filix acid with the fixed and volatile oils; Boehm [14] states that filix acid, if at all active as a tæniafuge, is much less so than albaspidin; Jaquet,[15] who has reported the latest work on the subject, concludes that the amorphous acid (filmaron) isolated by Kraft [16] is the active principle of the extract. The latter's views are corroborated by Stringari [17] and others. In as much as Kraft is the only investigator reporting the isolation of filmaron and as the method of isolation cannot be found in the literature, its identity as the chief anthelmintic constituent of the extract can hardly be said to be established.

[8] *Arch. d. Pharm.* (1852), 120, 87–89.
[9] Walko, *Deutsch. Arch. f. klin. Med.* (1899), 63, 348–358.
[10] Poulsson, *Arch. f. exp. Path. u. Pharm.* (1892), 29, 1–24.
[11] Gotthilf, *Münch. med. Wochenschr.* (1901), 48, 1096.
[12] *Loc cit.*
[13] *Chem. Centralbl.* (1893), 64, 269, from *Therap. Monatsh.* (1893), 7, 136.
[14] *Arch. f. exp. Path. u. Pharm.* (1897), 38, 35–38.
[15] *Jahresb. d. Pharm.* (1904), 64, 456, from *Therap. Monatsh.* (1904), 18, 391.
[16] *Pharm. Zeitg.* (1901), 48, 275–276.
[17] *Ibid.* (1910), 55, 426.

With the object of arriving at the therapeutic value of the male fern rhizomes and their extract, various methods for the estimation of the above-mentioned constituents have been devised. Some of them, as the methods of Bocchi [18] and Daccomo and Scoccianti,[19] or the method employed by Caeser and Loretz,[20] give results corresponding to the total amount of acid substances. (crude filicin) present; while the more specialized methods of Frommé [21] or Kraft [22] indicate only the filix acid [23] content. In view of the uncertainty regarding the chief tæniafuge constituent of the extract and the contradictory results obtained by various investigators (see above), the determination of the filix acid for the purpose of indicating the therapeutic value seems to be of little or no importance. The determination of the crude filicin in fresh green rhizomes or extracts prepared immediately from them undoubtedly serves, in a measure, as an index to their activity; but it serves neither to detect certain classes of adulterations in commercial extracts, nor to show the true therapeutic value of deteriorated extracts or those prepared from old rhizomes for reasons that will be considered later.

METHODS OF ADULTERATION

The adulteration of the extract is not limited to the addition of foreign substances to the finished product, but begins with the drug from which it is prepared. The forms in which the drug is contaminated may be conveniently classed under three heads: (*a*) the substitution of old deteriorated rhizomes for the fresh active drug, (*b*) the admixture of the chaff and dead stipe bases with the rhizomes, and (*c*) the addition of the rhizomes of other species of ferns to those of the official species. The first form of adulteration is, perhaps, the most common and most widely spread.

The pharmacopœia of the United States [24] directs that the dried rhizomes, from which the chaff together with the dead portions of rhizomes and stipes have been removed, and only such portions as have retained their internal green color, should be used. That it is almost impossible, from an economical standpoint, for the

[18] *Apoth. Zeitg.* (1901), 16, 233.
[19] *Pharm. Zeitg.* (1896), 39, 280, from *Boll. Chim. Pharm.* (1893), 130.
[20] *Jahresb. d. Pharm.* (1905), 65, 425.
[21] *Pharm. Centralhalle f. Deutschl.* (1897), 38, 34.
[22] *Schweiz. Wochenschr. f. Pharm.* (1896), 34, 217.
[23] Felix acid is used in this paper to represent the German "Filixsäure" rather than "filicic acid," the usual English translation, to avoid confusion with the "filicin acid" of Boehm.
[24] U. S. P., 8 Rev. (1905), 62.

American manufacturer to follow these directions becomes evident from the examination of various commercial samples. During the winter and spring of 1910, a number of commercial samples of male-fern rhizomes were examined in the pharmacy laboratories of the University of Wisconsin. These samples were purchased from importers and drug millers in different parts of the United States, and comprised specimens of the whole and peeled rhizomes. Only one sample showed the presence of the green rhizomes in considerable quantity, 53.7 per cent; the remainder varied from 0 to 18 per cent. One sample of 10 kilograms purchased from a drug miller in the Middle West was not male fern at all, but was identified as the rhizomes of *Osmunda*. The accompanying table shows the results of the examination:

TABLE I.—*Content of green rhizomes in samples of male fern purchased from drug millers and jobbers.*

Sample No.	Purchased in—	Content of green rhizomes.	Sample No.	Purchased in—	Content of green rhizomes.
		Per cent.			*Per cent.*
1	April	8.0	4	December	6.5
2	do	.0	5	do	18.0
3	do	53.7	6	do	*.0

* Contained rhizomes of *Osmunda* only.

During the present year, samples have been imported from the United States and Europe and examined in this laboratory upon their arrival. The samples were purchased in 1 kilogram lots. The parts showing an internal green color on breaking were separated from those showing an internal brown color. Table II shows the percentage of green rhizomes obtained.

TABLE II.—*Percentage of green rhizomes in samples of male fern.*

Purchased in—	Sample No.—	
	1.	2.
	Per cent.	*Per cent.*
United States	0.0	9.2
England	.0	.0
Germany	4.25	8.5
France	.0	.0
Manila, P. I.	.0	.0

The samples were not uniform, some consisting of the peeled rhizomes with stipe bases and chaff and, in one case, almost com-

pletely of stipe bases. Those purchased in Manila were at least 2 years old, and were badly deteriorated. The rhizomes purchased in Germany were obtained in January, and should have shown an internal green coloration had they consisted of the fresh stock harvested in the autumn; from this, it appears that the German supply is not renewed yearly as it should be, but is allowed to accumulate and deteriorate.

The use of old rhizomes cannot be detected in the extract by any of the previously mentioned assay methods as they show a crude filicin content equal to or greater than the fresh drug.

The greatest opportunity for adulteration is offered in the powdered rhizomes and, apparently, it has not been overlooked. The Belgian inspectors of pharmacies state that the powdered male fern, little used as such, is often superannuated and has completely lost its green color.[25] In many cases, the drug miller grinds up the entire rhizome including dead portions, chaff, and stipes. Rusby reports [26] a sample consisting of nothing but chaff and inert matter and another sample composed entirely of powdered *Osmunda* rhizomes.[27] It is not necessary, however, that the drug be powdered to permit of the addition of unofficial species of fern. Pendorff who [28] examined 20 samples of commercial rhizomes found that 12 contained 50 per cent or more of *Aspidium spinulosum* Sw.; 1 sample contained 90 per cent of the rhizomes of this species.

The latter form of adulteration may become evident from an assay by one of the analytical processes already mentioned, as the acid bodies present in other species of fern differ from those found in the official species both in their chemical constitution and in the quantities present. Hausmann [29] obtained the following results upon examination of the etheral extracts of *Aspidium filix mas* and *Aspidium spinulosum*.

TABLE III.—*Constitution of Aspidium filix mas Sw. and Aspidium spinulosum Sw.*

Species.	Crude filicin.	Felix acid.	Aspidin.
	Per cent.	*Per cent.*	*Per cent.*
Aspidium filix mas Sw	18.0	1.8	0.0
Aspidium spinulosum Sw	6.4	.0	1.1

[25] *Journ. d. Pharm. d'Anvers* (1909), 65, 550.
[26] *Pract. Drug.* (1910), 27, 423.
[27] *Drug. Circ.* (1910), 54, 616.
[28] *Apoth. Zeitg.* (1903), 18, 150–152.
[29] *Arch. d. Pharm.* (1899), 237, 544–556.

This investigator further affirms that the presence of aspidin in the extract is always evidence of the use of *Aspidium spinulosum* Sw. in its preparation. Taking into consideration the above observation of Hausmann, the report of Gehe & Co. of Dresden [30] is of interest. Upon the examination of 11 samples of the extract, 6 were found to contain aspidin, 2 to 3 per cent, but no filix acid; 4 samples contained filix acid, but no aspidin; and 1 sample showed a trace of aspidin and a small quantity of filix acid.

The adulteration of the finished extract consists in the addition of certain oils or foreign coloring materials. In the first instance, the only reason can be that of monetary gain. In the second case, the most plausible explanation is the desire to produce an extract from the deteriorated brown drug which will resemble that prepared from fresh rhizomes.

The oil usually employed in diluting the extract is castor oil, although others have been used. The quantity of oil added does not appear to bear any relation to the crude filicin content as 1 sample examined in this laboratory showed the presence of 54 per cent of castor oil and a filicin content of 8.79 per cent, while a second sample contained 62 per cent of oil and only 0.93 per cent of crude filicin.

This form of adulteration cannot be detected with certainty by any of the previously mentioned analytical processes as the crude filicin or filix acid content of the genuine extract prepared from the fresh rhizomes varies to such a great extent. Daccomo and Scoccianti [31] find the crude filicin present in quantities varying from 11.86 to 42.53 per cent, Bellingrodt [32] reports from 16.3 to 23.5 per cent present in commercial samples, while the Helvetian pharmacopœia [33] requires the presence of from 26 to 28 per cent. Madsen [34] reports the following concerning the filix acid content: Extracts from Bohemia and central Russia yielded from 0.71 to 0.97 per cent of filix acid by the Frommé method; Danish extracts with 2 exceptions (6.07 and 8.25 per cent) gave less than 2 per cent; 2 extracts from Germany showed the presence of from 6.58 to 9.59 per cent, respectively; and an extract from Wolmar in Livonia was found to contain 13.07 per cent. Furthermore, Kremel states that the adulteration with fatty oil cannot be detected by the saponification value, 1 gram of extract requiring

[30] *Pharm. Centralhalle f. Deutschl.* (1898), 39, 298.
[31] *Apoth. Zeitg.* (1896), 11, 174.
[32] *Ibid.* (1898), 13, 869.
[33] *Pharm. Helvetica*, IV Edit. (1907), 117.
[34] *Jahresb. d. Pharm.* (1897), 32, 591.

from 116 to 165 milligrams of KOH for saponification.[35] He proposes a solubility test instead.[36]

For the purpose of coloring the extract, two entirely different classes of substances have been made use of; namely, copper salts and chlorophyll. Neither class can be detected by the assay methods. The addition of copper can best be detected by the examination of the ash, applying the usual tests for copper. The use of copper salts seems to be very frequent, although their presence was not detected in the samples examined in this laboratory. Weppen and Luders [37] found 2 samples of a deep green color containing 0.056 and 0.044 per cent, respectively. Beckurts [38] reports 2 samples containing 0.135 and 0.044 per cent, and Pendorff [39] states that 7 of 20 samples examined contained more or less copper.

Chlorophyll is not very generally used for the artificial coloration of the extract, although 1 sample examined in this laboratory was highly colored with it and its use is reported in at least one other instance.[40]

DETERIORATION OF THE EXTRACT

As has been stated before in this paper, the extract owes its activity to various acid substances. Poulsson [41] considered the amorphous filix acid as being the most important of these. He further states that this acid may exist in 2 forms—the amorphous or active form and the crystalline or inactive form. Upon standing, the extract becomes weaker in its action as a tæniafuge owing to the conversion of the amorphous to the crystalline acid and its subsequent precipitation. Kraft,[42] in a later investigation of the subject, concludes that the principal constituent of the extract, from a therapeutic standpoint, is an amorphous acid, "filmaron;" this exists only in the amorphous form, and is not identical with the filix acid of Poulsson. However, he

[35] The saponification values obtained by Kremel have been found to be much too low for extracts prepared in this laboratory. An extract having a filicin content of 19.18 per cent gave a saponification value of 236.7.

[36] Kremel states that from 40 to 45 per cent of the pure ethereal extract is soluble in 95 per cent alcohol; less than 40 per cent means the addition of a fatty oil, more than 45 per cent going into solution means the addition of castor oil. *Pharm. Post.* (1887), **20**, 349.

[37] *Pharm. Zeitg.* (1893), **38**, 922.

[38] *Apoth. Zeitg.* (1893), **8**, 594.

[39] *Ibid.* (1903), **18**, 150–152.

[40] *Pharm. Zeitg.* (1893), **38**, 922.

[41] *Arch. f. exp. Path. u. Pharm.* (1892), **29**, 23–24.

[42] *Pharm. Zeitg.* (1903), **48**, 275–276.

states that the acid decomposes upon the aging of the extract forming "filix acid" and "filixnigrine." In either case, it will be noticed that the investigators have found a deterioration of the extract upon standing, a fact which is corroborated by numerous others. The only fact which has been definitely established is that the extract when freshly prepared is homogeneous, but upon aging it deposits filix acid among other substances and becomes weaker in its action as a tæniafuge. This behavior of the extract has been so universally observed that the various pharmacopœias give specific directions for the mixing of the deposit with the liquid portion before dispensing. Viewed in the light of the observations of Poulsson or Kraft, these directions are founded upon error and are superfluous. However, Greenawalt,[43] Reuter,[44] and others contend that the deposit is active. Here again, we are confronted with the uncertainty of the exact nature of the process of deterioration and the futility of attempting to estimate the therapeutic value of the extract by a determination of the crude filicin or filix-acid content.

In order to establish more definitely the physical and chemical properties of the genuine extract prepared from the green rhizomes, in contrast to those of the deteriorated or adulterated product, the following constants of the genuine and commercial samples have been determined and tabulated.

METHODS OF DETERMINING THE PROPERTIES

Color.—The color of the extract was observed with the naked eye when a few drops of the substance were allowed to flow down the side of a white porcelain capsule. The results are expressed in shades of green or brown as the case demands.

Specific gravity.—The specific gravity of the extracts was taken at 25° C. using a 5 cubic centimeter pycnometer and an ordinary chemical balance. Owing to the thick consistency of the substance special precautions had to be taken to eliminate all air bubbles. Warming the extract before filling the pycnometer has an advantage in this connection.

Refractive index.—The refractive index was observed at 15° C., using an Abbé refractometer. The results given in the tables are the averages of 5 readings, and are only given to the third decimal place as the deep color of the extract prevents a more exact reading.

Solubility.—The solubility tests were carried out at 15° C.; those with ether and acetone being exceptions as they were ob-

[43] *Am. Journ. Pharm.* (1889), 61, 169.
[44] *Pharm. Zeitg.* (1891), 36, 245–246.

served at room temperature, about 30° C. In each case, with the exception of ether and acetone, the specified quantities of the extract and solvent were placed in a 25 cubic centimeter graduated, glass-stoppered cylinder and thoroughly shaken. The mixture was then cooled for two hours at a temperature of 15° C., and the volume of the oil thus separated was noted. The difference in this volume and that of the original quantity of the extract represents roughly the amount of castor oil when used as an adulterant. Where no castor oil has been added, practically the original volume of the extract was observed. For a more exact determination of the castor oil, when present, the following procedure was carried out.

Quantitative determination of castor oil.—A weighed quantity of the extract, about 10 grams, was introduced into a separator and shaken with an equal volume of petroleum ether. Part of the petroleum ether was used to aid in transferring all of the extract from the container in which it was weighed to the separator. After shaking, the mixture was allowed to stand in the ice box until the separation was complete when the oily layer was drawn off. This process was repeated. The oil was then treated with a 2 per cent solution of sodium carbonate, washed with distilled water, and dissolved in ether. A small quantity of animal charcoal was added to the ethereal solution; it was then filtered into a 200 cubic centimeter Erlenmeyer flask and evaporated to constant weight on a water bath. It was identified by a determination of its physical and chemical constants.

Determination of the crude filicin.—The crude filicin was determined according to the method employed by Caesar and Loretz [45] which is essentially the same as that given in the Swiss pharmacopœia. The following is the procedure as carried out by this firm:

> Dissolve 5 grams of the extract in 30 grams of ether, add 100 grams of a saturated solution (3 per cent) of barium hydroxide, and shake the mixture vigorously during several minutes. Transfer to a separator, and run 86 grams (4 grams of the extract) of the lower aqueous layer into a flask of 200 cubic centimeters' capacity. Add 2 grams of hydrochloric acid, 25 per cent, and shake out with 3 portions of ether, 25, 15, and 10 cubic centimeters. Separate the ether, and filter each portion successively through the same plain double filter into an Erlenmeyer flask of 200 cubic centimeters' capacity which has been previously weighed. Wash the filter with 10 cubic centimeters more ether, and finally distill off the ether and dry the residue at 100° C. Weigh after allowing it to stand in a desiccator for half an hour. The weight multiplied by 25 will give the percentage of crude filicin in the sample.

[45] *Jahresb. d. Pharm.* (1905), 65, 425.

In addition to the above, the color of the ethereal solution of the crude filicin was carefully noted in each case.

Determination of aspidin.—The aspidin was determined as directed by Hausmann,[46] the crude filicin obtained in the above assay was dissolved in absolute ether, and the solution allowed to evaporate spontaneously when the aspidin crystallized in the form of fine needles. When it was not present, the solution merely thickened without depositing any crystals and finally hardened.

Determination of the iodine value.—The iodine value was obtained as directed in the United States Pharmacopœia.[47] Approximately 0.2 gram of the extract was used instead of 0.3 gram as directed.

Determination of the saponification value.—The United States pharmacopœial method for determining the saponification value of fats and oils[48] had to be modified somewhat as the solution, after saponification, was too highly colored to distinguish clearly the end point when titrating with phenolphthalein as an indicator. In order to overcome this difficulty, only about 1 gram of the extract was taken for the determination. The mixture after saponification was diluted with 50 per cent alcohol to 100 cubic centimeters in a volumetric flask; 25 cubic centimeters of this solution were transferred with the aid of a pipette to a 500 cubic centimeter Erlenmeyer flask, diluted to 300 cubic centimeters with distilled water, and finally titrated with half-normal hydrochloric acid.

Determination of the ash.—The ash was determined in the ordinary manner, heating carefully over a Bunsen flame until it assumed a grayish white color.

PROPERTIES OF GENUINE EXTRACTS

The physical and chemical constants of the genuine extracts are found to depend upon several factors; namely, the condition of the drug when extracted, the solvent used in exhausting the drug, and to some extent the method of preparation. Ether is the solvent generally employed for the extraction of the male-fern rhizomes, but as the eighth decennial revision of the United States Pharmacopœia specifies the use of acetone, the properties of the official extract have been determined and are here stated for the purpose of comparison. With respect to the method of

[46] *Arch. d. Pharm.* (1899), 237, 544–556.
[47] U. S. P., 8 Rev. (1905), 527–528.
[48] *Ibid.*, 535–536.

preparation, it has been found that a high temperature or prolonged heating causes the extract to darken somewhat in color.

The physical and chemical properties of extracts prepared from the drug in this laboratory are given in Tables IV, V, and VI. A commercial sample of 1,000 grams of the peeled drug was sorted, the rhizomes having an internal green color were separated from those having an internal brown color, and an extract was prepared from each lot using ether as the solvent.

TABLE IV.

Extract from—	Color.	Specific gravity, 25°.	Refractive index, 15°.
Green rhizomes	Yellowish green	1.0004	1.501
Brown rhizomes	Greenish brown	1.0048	1.490

TABLE V.

Solubility in—				
Ether.	Acetone.	Two volumes petroleum ether.	Three volumes 90 per cent alcohol.	One volume glacial acetic acid.
Soluble	Partially soluble	Mixes[a]	Oil separates	Oil separates.
Do	do	do	do	Do.

[a] The term "mixes" is to be interpreted as meaning the formation of a turbid solution without the separation of oily particles.

TABLE VI.

Extract from—	Crude filicin.	Iodine value.	Saponification value.	Ash.
	Per cent.	*Per cent.*		*Per cent.*
Green rhizomes	19.98	99.3	224.8	0.48
Brown rhizomes	20.64	101.7	237.2	.49

It will be noticed upon examination of Table VI that the iodine and saponification values vary directly as the filicin content. Such a variation, however, is not constant for extracts prepared from different samples of the drug, owing to the varied composition of the crude filicin and to the variable quantities of the fixed [49] and volatile [50] oils present. Another important item

[49] Wollenweber states the fixed oil constitutes from 70 to 75 per cent of the extract. *Arch. d. Pharm.* (1906), 244, 466.

[50] Ehrenberg found the content of volatile oil to vary with the season in which the rhizomes were collected; those gathered in September yielded 0.04 per cent of volatile oil, those gathered in April 0.008 per cent, and those gathered in June 0.025 per cent. *Arch. d. Pharm.* (1893), 231, 345–356.

brought out in the above table is the fact that the brown rhizomes yield an extract richer in filicin than the green. The higher filicin content of the brown rhizomes can probably be attributed to the breaking down of glucosidal bodies [51] with the liberation of acids, or it may be due to a slight variation in the filicin content of the rhizomes themselves. (See previous discussion under adulteration with oils.)

The influence of the solvent used in the preparation of the extract upon its physical and chemical properties is set forth in Tables VII, VIII, and IX. In contrast with the ether preparation, it was noticed that the acetone extract separated into two layers, an upper oily layer having a brownish green color and a lower layer which was nearly brown and thicker than that above. Both of the extracts were prepared from the rhizomes having an internal green color. The two extracts can be distinguished by their color, specific gravity, refractive index, and solubility.

The low percentage of filicin in the acetone extract is due to the greater yield of extractive matter when acetone is used as a solvent and not to the incomplete extraction of the acid bodies as might be inferred from the table. Ether yields an extract amounting to 8.325 per cent of the drug while acetone produced 14.690 per cent.

TABLE VII.

Extract.	Color.	Specific gravity, 25°.	Refractive index, 15°.
Ether	Yellowish green	1.0008	1.500
Acetone	Brownish green	1.0480	too dark

TABLE VIII.

Solubility in—				
Ether.	Acetone.	Two volumes of petroleum ether.	Three volumes of 90 per cent alcohol.	One volume of glacial acetic acid.
Soluble	Partially soluble	Mixes	Oil separates	Oil separates.
Partially soluble	Soluble	Partially soluble	do	Do.

[51] Penndorff attributes the turning brown of the green rhizomes to the breaking down of the "filix tannic" acid into "filix red" and sugar. *Loc. cit.*

TABLE IX.

Extract.	Crude filicin.	Iodine value.	Saponification value.	Ash.
	Per cent.			*Per cent.*
Ether	20.37	99.8	229.3	0.48
Acetone	13.79	95.3	208.5	.63

PHYSICAL AND CHEMICAL CONSTANTS OF COMMERCIAL EXTRACTS AS DETERMINED IN THE LABORATORY

The commercial extracts were purchased in different countries and subjected to the same tests as the genuine extracts prepared in the laboratory. Their properties are shown in Tables X, XI, and XII.

TABLE X.

Sample No.	Color.	Specific gravity, 25°.	Refractive index, 15°.	Source.
1	Deep green	1.0079	1.490	United States.
2	Brownish green	1.0085	1.494	Germany.
3	do	1.003	1.493	England.
4	do	1.0028	1.492	Germany.
5	do	1.0012	1.490	United States.
6	do	.9889	1.485	England.
7	do	.9855	1.484	Do.
8	do	.9773	1.489	Manila, P. I.

TABLE XI.

	Solubility in—				
Sample No.	Ether.	Acetone.	Two volumes of petroleum ether.	Three volumes of 90 per cent alcohol.	One volume of glacial acetic acid.
1	Soluble	Partially soluble	Mixes	Oil separates	Oil separates.
2	do	do	do	do	Do.
3	do	do	Partial separation.	Partial separation.	Mixes.
4	do	do	Mixes	Oil separates	Oil separates.
5	do	do	do	do	Do.
6	do	do	Partial separation.	Partial separation.	Mixes.
7	do	do	do	do	Do.
8	do	do	Mixes	Oil separates	Oil separates.

TABLE XII.

Sample No.	Crude filicin.	Iodine value.	Saponification value.	Ash.	Adulterant.
	Per cent.			*Per cent.*	
1	20.77	101.5	240.5	0.49	Chlorophyll.
2	20.32	100.2	225.5	.47	
3	17.61	98.3	208.7	.42	Castor oil.
4	16.55	97.1	214.6	.48	
5	14.36	94.4	206.7	.47	*A. spinulosum.*
6	8.79	89.4	202.4	.27	Castor oil.
7	0.93	85.8	195.7	.18	Do.
8	.59	87.2	200.3	.37	

An inspection of the above tables indicates the use of old brown rhizomes in the preparation of the extracts. This is especially prominent in the color and refractive indices. Further evidence of this is found in the color of the ethereal solution of the crude filicin obtained in the filicin assay. All of the above extracts yielded a product which dissolved in ether with a color varying from light brown to dark reddish brown. The color of the ethereal solution of filicin obtained from fresh green rhizomes, although varying somewhat, was not of a darker shade than orange.

Extract 1 contained a considerable quantity of chlorophyll. Because of the fact that chlorophyll is a natural constituent of the extract and that there is no satisfactory method for its quantitative estimation, a determination of the amount present was not attempted. Its presence in undue quantity was based on the deep green of the preparation and the green color imparted to alcohol when shaken with a portion of the sample.

Extracts 3, 6, and 7 contained 30, 54, and 62 per cent of castor oil, respectively. This fact is indicated by the refractive index, the specific gravity, the iodine and saponification values, and the ash content.

Another fact of interest to be noted in the above tables is that the iodine and saponification values vary in the same direction as the filicin content. The one exception to this is found in sample 8. Upon an examination, however, of the physical and chemical properties of the fixed oil of male fern and those of castor oil, the apparent irregularity is readily accounted for. Tables XIII, XIV, and XV show constants of the fixed oil of fern separated from an extract prepared in the laboratory and of castor oil as specified in the United States Pharmacopœia.[32]

[32] U. S. P., 8 Rev. (1905), 321.

The index of refraction of castor oil is that given by Lewkowitsch.[33]

The fixed oil of fern was obtained as follows: The extract was treated with 5 volumes of a 2 per cent solution of sodium carbonate, and the fatty oil shaken out of the mixture with several portions of petroleum ether. The petroleum-ether solution was washed with distilled water in a separator, the aqueous layer drawn off, and a small quantity of animal charcoal added to the remaining solution. The mixture was filtered into a tarred porcelain dish and evaporated to constant weight on a water bath.

TABLE XIII.

Oil.	Color.	Specific gravity, 25°.	Refractive index, 15°.
Oil of male fern	Brownish yellow	0.921	1.4773
Castor oil	Pale yellow	0.945 to 0.965	1.4795 to 1.4803

TABLE XIV.

Solubility in—		
2 volumes of petroleum ether.	3 volumes of 90 per cent alcohol.	1 volume of glacial acetic acid.
Soluble	Insoluble	Insoluble.
Insoluble	Soluble	Soluble.

TABLE XV.

Oil.	Iodine value.	Saponification value.
Oil of male fern	[a] 118.19	197.51
Castor oil	[b] 86 to 89	179–180

[a] Four hours. [b] Eight hours.

SUMMARY AND CONCLUSIONS

The fact that the present methods of assaying the extract of male fern do not give results which indicate its therapeutic value has already been brought to notice. Neither can the properties of the extract tabulated in this paper be used as factors for indicating the degree of activity. They can only serve the physician and pharmacist in so far as they afford a means of

[33] Chemical Analysis of Oils, Fats and Waxes. Macmillan & Co., N. Y. (1898), 423.

distinguishing between an extract recently prepared from fresh green rhizomes and that prepared from old rhizomes or in detecting a deteriorated or adulterated product. Viewed from this standpoint the following conclusions can be drawn.

The color of the extract when prepared from fresh rhizomes is yellowish green; a brownish shade indicates the use of old rhizomes; deep green means the addition of chlorophyll or other foreign coloring material, such as a salt of copper. The use of old rhizomes receives further confirmation in a low index of refraction (the refractive index is also lowered by the addition of castor oil) and in the dark color of the ethereal solution of the crude filicin obtained in the filicin assay. The presence of a copper salt can best be confirmed by an examination of the ash, applying the usual tests for copper. The addition of chlorophyll is difficult to detect chemically owing to its presence as a natural constituent of the extract. However, the amount which must be added to obscure the brown color imparted by old rhizomes is so great that it can be easily detected with the naked eye in the original sample and in the solubility tests.

The addition of castor oil to the extract produces a considerable change in all of its properties. It is especially indicated in the low specific gravity, index of refraction, and iodine and saponification values. Its presence can be most easily confirmed by the solubility tests.

The presence of aspidin can readily be determined by Hausmann's method which serves as a practical means of detecting the use of *Aspidium spinulosum* Sw. in the preparation of the extract.

The use of acetone as directed in the United States Pharmacopœia yields a product which separates in two layers upon standing—an upper oily layer and a lower layer darker in color and thicker in consistency than that above. This in itself serves as a ready means of detecting its use, which is further confirmed by the solubility tests and the high ash content.

The iodine and saponification values vary in the same direction as the filicin content, and, therefore, might be used to displace the rather long and expensive method of the present filicin assay. Especially is this true in the case of the saponification value, which requires but 1 gram of the extract and less than an hour to complete.

THE VARIABILITY OF CERTAIN STRAINS OF DYSENTERY BACILLI AS STUDIED BY THE SINGLE-CELL METHOD

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One plate and 1 text figure

During the summer of 1912, a considerable number of stools from cases of bacillary dysentery occurring in Manila came into the laboratory. Some of the cultures from these stools showed a tendency to variability, and it was decided to study some of these variations by the single-cell method.

This method, described by me in previous publications,[1] enables the worker not only to obtain easily pure cultures arising from a single cell, but also to select any aberrant cell lying among myriads of normal ones and to substitute the single-cell cultivation for plate cultures in obtaining a series of selections. Further, one may more easily avoid the contaminations which are especially liable to occur in the Philippine Islands when plate cultures are used. A description of the technique is given below. The difficulties of using the method are not great. Pure cultures may be made at the rate of 50 per hour under favorable circumstances.

Two types of variations were studied; one characterized by fermentative, the other by morphological, characteristics.

I. FERMENTATIVE CHANGES WITH RESPECT TO MALTOSE

A culture, laboratory strain 105200, furnished by Dr. Otto Schöbl of this Bureau, was isolated July 7, 1912, from a typical bloody stool of a case of bacillary dysentery. This strain was mannite fermenting, and otherwise showed the characteristics of dysentery bacilli of the Flexner type. A pure culture was made by the isolation of a single bacillus, and this culture was grown on ordinary agar for about eighteen days. Transfers were made on September 12, 1912, to ordinary broth, and allowed to grow from two to five hours, in order to obtain actively growing

[1] *Sci. Bull.*, Kansas Univ. (1907), 4, 3; *Journ. Infect. Dis.* (1908), 5, 380; (1909), 6, 634.

bacilli. Twenty-one single cells were isolated and allowed to develop overnight in hanging drops, and were then transferred to ordinary agar. After twenty-four hours' growth, they were transferred to maltose-litmus agar and incubated. Of the 21, 16 gave cultures like the original stock; that is, with blue surface growth and, at most, only slight acid formation at the bottom; while 5 gave within twenty-four hours the practically complete cherry-red color to the agar, such as is found in any maltose-fermenting organism. After twenty-four days at room temperature, 2 of the 5 red cultures remained clear red, 2 showed the lower two-thirds of the tube red with the upper part becoming blue, and the fifth had become almost wholly blue. The 16 originally blue cultures showed a deep blue, deeper than the uninoculated controls. Transfers from the originally red cultures, that had now become blue, to fresh maltose agar gave distinct red cultures like the original; while transfers from the blue gave blue cultures. So, in spite of the later change to blue through long exposure to the air, the red cultures possessed a distinct fermentative difference from the blue in that they showed complete red in forty-eight hours; and, although becoming blue later, gave a distinct red color on transfer to a new maltose-litmus medium. The change of the red coloration to blue on long exposure was not constant. In some whole series the agar remained red for weeks. Some slight variations in the composition of the medium may account for the differences. It was found that cultures remaining acid were more likely to die out.

A further characteristic of the original blue variety is the property of forming on maltose agar secondary colonies capable of fermenting maltose. These colonies are somewhat elevated, denser than the surrounding growth, and usually well defined in the substratum. They often appear within forty-eight hours, sometimes increasing in number during subsequent days, and, in this series, varying from 1 to 300 per test tube. They often increase in size, and may become 4 millimeters or more in diameter. At first they show no color, but later change the agar at their base to a dull red, and if numerous may redden the whole tube. If a transfer is made from one of these colonies to a new tube, a permanently acidifying growth is obtained; while a transfer from the intervening substratum gives a blue culture like the original.

Many references are found in the literature to secondary colonies of this sort ("papillae," "Knöpfe," "Knötchen") possessing new fermentative characteristics. They were described

by M. Neisser[2] as occurring in *Bacterium coli mutabile*, the secondary colonies of which had the property of fermenting lactose. The secondary colonies of *B. coli mutabile* were further studied by Massini,[3] Arnold Burk,[4] Sauerbeck,[5] Kowalenko,[6] Baerthlein,[7] and Klein.[8] Burri[9] found similar secondary colonies in *Bacterium coli inperfectum*. Burri and Düggeli[10] and Hübener[11] found them in various strains of *Bacterium coli* or coli-like organisms. R. Müller[12] reported secondary colonies in many different kinds of bacteria on 18 different varieties of carbohydrate media.

In a typhoid-like organism isolated from urine, Sobernheim and Seligmann[13] found secondary colonies fermenting lactose in Conradi-Drigalski agar. Penfold[14] obtained in *Bacillus typhosus* secondary colonies which fermented dulcite. Jacobsen[15] found secondary colonies in Conradi agar plates made of an atypical strain of typhoid (*Bacterium typhi mutabile*), which had caused a typhoid epidemic in Denmark. The original strain varied from typical typhoid bacilli in that it possessed a very low degree of agglutinability to typhoid serum and a delayed acid formation in mannite broth. The secondary colonies gave cultures which grew unrestrained on Conradi agar and possessed the agglutinability and fermentation characteristic of typhoid bacilli. Seiffert[16] worked with a strain of *Bacterium coli* which had been made resistant to malachite green and had lost the power of forming red colonies on Endo agar. Secondary colonies were obtained from this modified strain which not only had regained the power of producing red cultures on Endo agar, but also exhibited a character not possessed by the original strain—that of fermenting cane sugar.

In the dysentery and paradysentery group of bacilli, R.

[2] *Centralbl. f. Bakt. etc.*, Ref. (1906), **38**, Beiheft, 98.
[3] *Arch. f. Hyg.* (1907), **61**, 250.
[4] *Ibid.* (1908), **65**, 235.
[5] *Centralbl. f. Bakt. etc.*, Orig. (1909), **50**, 572.
[6] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1910), **66**, 277.
[7] *Centralbl. f. Bakt. etc.*, Orig. (1912), **66**, 21.
[8] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1912), **73**, 87.
[9] *Centralbl. f. Bakt. etc.*, II Abt. (1910), **28**, 321.
[10] *Centralbl. f. Bakt. etc.*, Orig. (1909), **49**, 145.
[11] *Centralbl. f. Bakt. etc.*, Ref. (1909), **44**, Beiheft, 136.
[12] *Münch. med. Wochenschr.* (1909), **56**, pt. 1, 885.
[13] *Centralbl. f. Bakt. etc.*, Ref. (1911), **50**, Beiheft, 134.
[14] *Journ. Hyg.*, Cambridge (1911), **11**, 30.
[15] *Centralbl. f. Bakt. etc.*, Orig. (1910), **56**, 208.
[16] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1912), **71**, 561.

Müller [17] describes a strain of the Flexner type which produced secondary colonies capable of fermenting isodulcite.

Bernhardt [18] investigated a strain of dysentery bacilli, type Y, which formed secondary colonies on maltose agar capable of fermenting maltose. The new characteristic was transmissible when cultures were transferred at short intervals, but, after eight weeks' growth on weakly alkaline agar or after half a year in sealed agar tubes, the power to acidify maltose was lost. On transferring to fresh agar, new secondary maltose-fermenting colonies were formed.

Baerthlein,[19] in an extensive article on the so-called mutations in various bacilli, vibrios, and cocci, describes secondary colonies in strains of dysentery bacilli of both the Shiga-Kruse and of the nontoxic varieties. Secondary colonies possessing new morphological as well as fermentative qualities were obtained, although there was no constant correlation between the morphological and chemical changes. As a rule, secondary colonies were formed on transfer of old agar cultures to new media. Some mutants of the same strain of toxin-free dysentery bacilli possessed varying agglutinability to the same serum. In some cases the same strain gave two or more sorts of mutation.

Practically all of the above-mentioned writers obtained secondary colonies only in media containing the sugar fermented by the new race, and in nearly all cases the new race transmitted its characteristics indefinitely to offspring. In most cases the purity of the cultures used was established by plate cultures only, but in some the varying strain was started from a single bacillus obtained by the Burri method.

In working out the maltose-fermenting variety of dysentery bacilli observed by me, it was proposed to examine the variations carefully, not only beginning with a single cell, but testing numerous individuals, both of the stock and of the new races, by this method. It was recognized that dysentery bacilli of the Flexner type are relatively inconstant in their characteristics, and that maltose is a comparatively unreliable substance with regard to fermentation; but the characteristics of the races obtained were so well marked and constant, that they could be safely taken as a basis for a study primarily of variation.

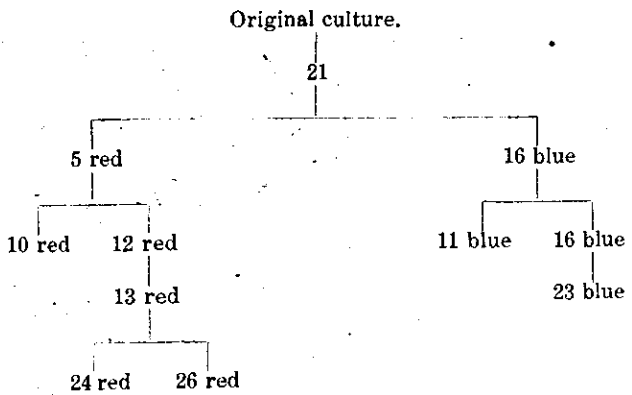
First, the uniformity of the individuals of the red and the blue

[17] *Centralbl. f. Bakt. etc.*, Ref. (1908), 42, Beiheft, 57.
[18] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1912), 71, 229.
[19] *Arb. a. d. kais. Gesundheitsamte* (1912), 40, 433.

types was tested by selecting individuals at random from each type and testing the characteristics of their offspring.

From a culture of the red, or maltose-fermenting, series, 10 single cells were isolated and their offspring tested on maltose agar. All gave the characteristics of the red variety. From another one of the original 5 red cultures, 12 isolations gave red cultures on maltose agar. After an interval of four days, 13 new isolations were made from one of the 12, selecting a tube which gave a deeper red than the average. All gave red cultures. A subculture of one of these 13 showed a tendency to blue, and some days later 24 new isolations were made from it, and at the same time 26 isolations were made from one of the same lot which showed a deeper red than the average. All 50 of these gave cultures which were alike red. Apparently selection in the direction of red or blue in this red series showed no progression toward either color, and it is probable that the slight variations in color were due to some inequalities in the medium.

From one of the original 16 blue, 11 cells were selected, all giving cultures of the blue type. From another one of the original 16, 16 single cells were isolated and gave blue cultures, and from one of this second group 23 isolations were made and all gave a similar blue type. All blue cultures gave secondary colonies fermenting maltose. No red cultures showed such colonies. The selections are given graphically in the chart below:



During the period of these isolations the stock cultures were kept on maltose agar except shortly before isolations, when they were transferred to ordinary broth to obtain actively growing cells for isolation. In obtaining material for selection from the blue type, transfers were made from growths between sec-

ondary colonies. Only cells of apparently normal morphology were selected. All of the above series of selections were made within a month after the original selection.

About five months after the first selections from strain 105200, a new lot of cells was selected from it. The culture had been kept on ordinary agar at room temperature with occasional transfers to keep it viable. It had never grown on a maltose-containing medium. Of 60 isolations, 52 gave typical blue cultures, 6 typical red, while 2 gave partial red with no secondary colonies. To test the composition of one of the partially red strains, 9 single cells were isolated from it, and, as a control, 13 from one of the typically red cultures. The cultures from the 9 of mixed type gave all mixed color with no secondary colonies, while the 13 red gave all pure red cultures.

Nearly ten months after the first series of selections a new lot of single cells was isolated from the original stock of dysentery strain 105200. The culture had been kept at room temperature on ordinary agar. It had been transferred at about monthly intervals to fresh agar, and had had in all about 10 transfers since the hanging drop in which the original single cell had been isolated. In this third series 123 cells were isolated, and the cultures grown from them tested on maltose agar, before a maltose-fermenting type was found. This, however, was typical, exhibiting a clear red color throughout the whole tube within twenty-four hours after sowing. No secondary colonies were formed. Of the 122 nonfermenting strains, all formed secondary colonies; in some very few and in others so many as to redden the whole tube. However, there was no possibility of confusing such cultures with a "red" variant, since the color appeared in the former only after several days and after a very large number of secondary colonies had been formed. No partially red strains were found, such as occurred in the second series.

In this third series different lots were selected at a time, the selections extending over a period of some days. In each lot of selections a fresh broth culture was made from the original agar culture in order to obtain freshly growing cells for isolation.

So, in all series, 205 isolations were made, and from these 11 (about 5 per cent) of the red variants were obtained.

Either the two varieties exist side by side for months on ordinary agar, or the maltose-fermenting variations are being continually formed from the stock. The proportion of acid formers is the smaller, 5 in 21 in the first series, 8 in 60 in

the second, and 1 in 123 in the third—the proportion becoming smaller as the months went on. In addition, there occurred varieties with less power of fermenting maltose, but otherwise corresponding to the acid type.

As a further test of the constancy of an acid-forming strain, single cells were isolated from a red strain which had been cultivated two months on ordinary agar at room temperature. It had been passed through 5 subcultures, and neither it nor the culture from which it was isolated had been grown on maltose agar. Twenty-nine such isolations gave cultures all of which completely fermented maltose-litmus agar.

In order to test the behavior of the red and blue varieties in ordinary broth alone and in competition with each other, the following experiment was arranged:

Into 4 tubes of ordinary broth, A, B, C, and D, equal amounts were sown of the red and blue strains. In order to have exactly equal sowings of the two varieties, the following method was followed: Single cells of each of the two types were isolated and grown from one to two hours in hanging drops until 3 generations had formed, that is, until 8 new individuals had formed from one. Then to a tube of ordinary broth 8 bacilli of the red and 8 of the blue strains were added, selecting such hanging drops as showed elements of approximately equal size. This was done in 4 tubes and the fifth, E, was given 5 bacilli of the blue and 6 of the red variety. For comparison, a tube was sown with a pure culture of the red and one with a pure culture of the blue type. These tubes were incubated one day, then transfers made by platinum loop to fresh broth and at the same time to maltose agar, in order to observe any fermentative changes. This was continued through 10 daily transfers.

At the end of the series, the red control remained red as before, and the blue distinctly blue with secondary colonies; while of the 4 which received mixed sowing in equal quantities, 1, A, was blue with secondary colonies like the blue control, 2, B and C, showed mixed red and blue, while the fourth, the one which had received a slightly larger amount of red strain, was as red as the red control. In order to test the composition of the cultures, a new transfer was made at the end of the series to ordinary broth to obtain freshly growing cells, and 19 single cells were isolated from A of the mixed series, a culture which showed all blue. All 19 gave typical blue colonies. Ten similar isolations from D, which showed a mixed coloration on maltose agar, gave 8 of the red and 2 of the blue; 12 isolations from the

pure blue, which had been carried through 11 daily transfers on bouillon, gave all typical blue cultures with secondary colonies.

This experiment indicates: First, that with daily transfer in broth the pure types remain pure; and, secondly, that in competition with the blue the red tends to gain the upper hand, although in 1 tube the blue predominated and in 2 tubes the red and blue persisted side by side.

After the one-day incubation, all broth tubes of this series were kept at room temperature. In order to ascertain the varying proportions of the red and the blue strains at different stages of the experiment, samples were taken from the first, fourth, and eighth transfers of A, and put into broth to get actively growing cells. From these, single cells were isolated and their descendants tested on maltose agar. Of 13 cells of the first transfer, 7 gave blue and 6 red cultures. Of 7 cells from the fourth transfer, 6 gave blue and 1 red cultures. Of 15 from the eighth transfer, all gave blue.

For confirmation, a second mixed series in ordinary broth was undertaken. Here 4 tubes were given equal mixtures by the method described above and 1 by simply adding a loopful of each type. Two pure red and 2 pure blue cultures were added to the series, and daily transfers and daily tests made as in the previous experiment. The same batch of maltose agar was used in all tests. On account of an enforced absence from the laboratory, the experiment had to be interrupted two weeks, between the first and second transfers, but all were kept in the refrigerator during the interval, and in a comparative experiment this interruption ought not to vitiate the results.

The blue and the red controls retained their characteristics after 10 transfers; and, of the mixed ones, 3 became acid at the fifth; 1, the tube inoculated with the loop mixtures, at the seventh; and 1, B, showed mixed red and blue at the tenth transfer. Twenty-one single-cell isolations from B gave 19 blue and 2 red cultures. Nineteen isolations from A, a tube which had received an equal mixture of the blue and red strains and had become red, gave 19 all red cultures.

In a third mixed series, all were passed through daily transfers in three kinds of broth—plain, maltose, and mannite. The sugar broths were made by adding to water 1 per cent peptone, 0.5 per cent salt, and 1 per cent of mannite and maltose, respectively. The bacterial mixtures were made by adding to a broth tube equal quantities (about 0.25 cubic centimeter) of each

type. The mixed tubes were done in triplicate, 3 tubes to each kind of broth. The unmixed red and blue strains retained their characteristics through 15 daily transfers in each kind of broth. Of the 9 mixed tubes, all showed mixtures of red and blue strains at the end of the fifteenth transfer, except 2, 1 maltose and 1 mannite, which became distinctly acid at the fifth transfer.

These three series indicate that, whether in plain, mannite, or maltose broth, the characteristics of the two types remain constant if daily transfers are made. If the two strains be equally mixed in the same tube, sometimes the red and sometimes the blue gets the upper hand, but there is a tendency for the red strain to predominate.

Most authors have found that a new fermentative character is acquired only in contact with the corresponding sugar and that older cultures in the sugar have given more pronounced variation than fresh cultures after repeated transfers at short periods. The older cultures afford the time necessary for the exhaustion of the preferred sources of nutrition, after which the bacteria may acquire the power of attacking the unusual food. Baerthlein [20] found that a paratyphoid-like race of bacilli, grown on lactose-containing media, kept its characteristics when transferred every twenty hours, but, transferred at somewhat longer intervals, it acquired the power of acidifying lactose. Josef Klein,[21] working with *Bacterium coli mutabile,* found that many generations in a lactose-free medium did not produce the lactose ferment, while relatively few generations in a medium poorly supplied with nourishment, but containing lactose, brought about lactose fermentation. Mere contact with lactose at a temperature too low for active multiplication failed to develop the lactose ferment. Twort [22] succeeded in developing the power of fermenting dulcite and lactose in a strain of typhoid by fortnightly transfers in a medium containing these substances. Hiss [23] found that a strain of dysentery, type Y, cultivated for some time in maltose, had acquired the power of fermenting that sugar. On the other hand, Lentz [24] describes a Flexner strain which, after seven years' cultivation, lost the power to ferment maltose, while retaining its specific agglutinating power.

[20] *Centralbl. f. Bakt. etc.,* Orig. (1912), 66, 21.
[21] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1912), 73, 87.
[22] *Proc. Roy. Soc. London* (1907), B, 79, 329.
[23] *Journ. Med. Research* (1904), 13, 36.
[24] Kolle und Wasserman, *Habk. Handbuch der pathogenen Mikroorganismen. Gustav Fischer, Jena* (1909), Ergänz-Bd. 2, 407.

Penfold [25] succeeded in "training" typhoid to increased fermentative power in isodulcite.

In the race of dysentery bacilli experimented on by me, it is evident that contact with maltose is not necessary to the formation of the maltose ferment, since an ordinary agar culture of a race which had never been in contact with maltose gave, of single isolated bacilli, about 25 per cent of maltose fermenters, approximately sixty-five days after isolation from the patient, and about 13 per cent after culture on agar for five months. It may be that the agar used contained some substance which might replace maltose as an excitant of a maltose ferment, but it is more probable that in this case variations in fermentative character occur without a special food stimulus. Again, in this race of dysentery, growth in contact with maltose did not materially increase the proportion of maltose fermenters, unless kept for a long time in the same tube. Several series of experiments were carried out to test this matter.

In series 1, an alkaline and an acid-forming race were carried through 15 successive daily transfers on 3 sorts of broth: the first containing 1 per cent peptone, 0.5 per cent salt, and 1 per cent maltose; the second having the same ingredients with mannite instead of maltose; and the third consisting of ordinary beef-peptone broth. Cultures were tested on litmus-maltose agar slopes. At the end of the series the acid remained acid as before and the alkaline remained alkaline with no increase in the proportion of secondary maltose-fermenting colonies.

In a second series, an alkaline race, which had never grown on a maltose medium, was cultivated fifteen days; in one lot with daily transfers and in the other with transfers at intervals of three days, a period of time sufficient for allowing the formation of secondary colonies on maltose agar. Six different media were used: One, a broth containing peptone, 2.5 grams; sodium phosphate, 2.5 grams; calcium chloride, 5 cc.; asparagin, 5 cc.; maltose, 10 cc.; water, 1,000 cc.; 2, the same as 1, plus enough agar to make a soft medium; 3 and 4 the same as 1 and 2, respectively, but containing no sugar; 5, ordinary beef-broth peptone; 6, ordinary agar. Cultures were tested daily on maltose-agar slopes. At the end of the series both lots, the one transferred daily and the one transferred every three days, remained the same as at the start, except that the ordinary agar one showed some, although not a constant, tendency to become more acid. Since there was some suspicion that the

[25] *Journ. Hyg.*, Cambridge (1911), 11, 30.

maltose used contained a trace of glucose, a third series was carried out, using the same media as in the second but in addition a broth and an agar made with a maltose shown by chemical test to be pure. Ten daily transfers, and 3 at intervals of three days, failed to alter the character of the race.

In addition, tests were made of the above cultures after incubating four, seven, fifteen, and nineteen days. Here a tendency to an increase of acid producers was noted in the maltose media, while in the sugar-free media the strain remained unchanged.

In summary, it may be said that there is evidence that in this race of dysentery bacillus the presence of maltose does not materially increase the proportion of maltose fermenters except after long contact with the sugar in the same tube.

To compare the effect of substances other than maltose, a typical red and a typical blue race were sown on slants of the following litmus agars: Glucose, levulose, lactose, saccharose, raffinose, glycerine, dextrin, inulin, salacin, erythrin, and dulcite. All tubes were alkaline after three days' incubation except glucose, levulose, and glycerine, which were cherry red in both the red and the blue races. After seven days' growth, each culture was transferred to a new agar of the same kind. The colors remained the same as after the first transfer. On transfer back to maltose, the originally red strain gave red cultures in all cases and the originally blue strain gave blue. Apparently growth on other substances does not affect the fermentative character of the two races with respect to maltose; and, in a short time, at least, neither race acquires the power of attacking a new substance.

For confirmation, a red and a blue strain, neither of which had been grown on a medium containing maltose or other sugar, were sown on the media used in the first test, and, in addition, on mannite, galactose, and amygdalin-litmus agars. Both races gave the same reaction on all media.

Some further experiments were made to determine the composition of the secondary colonies. These colonies have a large proportion of irregular cells, many of which are coccoid or resemble in form small yeast cells. Many of these cells were isolated and grown to determine their nature. One protocol may serve to illustrate the nature of these experiments:

From the top of a large secondary colony eight days old, 10 normal cells were isolated, of which 3 grew; and 14 coccoid forms, of which 6 grew. Grown in hanging drops of ordinary

broth, 2 of the 3 normal gave nearly normal offspring, while 1 gave a large proportion of coccoid forms. Of the 6 coccoid cells, 1 gave a large proportion of coccoid, 2 gave a small proportion of coccoid cells, and 3 nearly regular offspring. Transferred to ordinary agar and broth, all 9 gave cells with uniform, normal morphology. Sown on maltose-litmus agar, all 3 cultures of normal-cell ancestry gave acid types, and of the coccoid strains 2 gave acid and 5 alkaline cultures.

From a secondary colony of three days' growth similar to the above, cells were spread over the surface of maltose-litmus agar in Petri dishes. Forty distinctly blue and 6 bright red colonies were obtained.

From the above and similar experiments, it was proved that the secondary colonies contain a mixture of acid- and alkaline-forming cells and that there is no constant correlation between the morphology or degree of involution of cells and their fermentative properties. Further, it was shown conclusively that the first few generations from an involution cell may show a large proportion (in some cases nearly one-half) of involution cells. Later, the morphology of the cells becomes normal or nearly so.

In an ordinary transfer from a secondary colony to fresh maltose-agar one usually obtains an acid-forming growth, although in some cases not so well marked as when a "pure" acid strain is sown. It is probable that in this mixture, taken from a secondary colony, the acid formers usually outgrow the others, so as to give a more distinct red to the maltose-litmus agar.

With regard to agglutination, the parent stock and the red and blue races isolated from it gave practically the same results. A serum obtained by inoculating a rabbit with a pure culture of the stock gave agglutination to $\frac{1}{800}$ in all three strains.

II. VARIATION IN MORPHOLOGY

In another culture of dysentery, No. 42 of the Shiga-Kruse type, a variation of a different kind was studied. This culture was kindly furnished me by Dr. C. S. Butler of the United States Navy, August 19, 1912, and came from a stool of a case of dysentery. A pure culture was obtained by isolating a single cell, August 30, 1912. Descendants from this normal cell grown in hanging drops showed, for the most part, only normal cells. From this pure culture a single cell was again isolated and grown

in a hanging drop on September 9. After twenty-four hours' growth, among many thousands of normal cells, some were found which grew chain-like with elements much more plump than the normal. Six of these variants were isolated, and of the six 1 grew. This, transferred to agar, grew apparently normally, but the cells showed a morphology decidedly different from that of the parent type (Plate I).

For confirmation, various other similar races were isolated from the stock, and all showed the same general characteristics. Not only was the morphology of cells grown on agar different, but, when transferred to ordinary bouillon, adherent masses, instead of the usually separate elements, were formed. These masses, sinking to the bottom, left the supernatant fluid clear, while the controls remained nearly uniformly cloudy. After cultivation at room temperature for over one year and passage through 54 agar transfers, the clumping tendency was as marked as before. The irregularity in the form of the cells became less marked after some months, and finally the culture became in this respect like the control.

The new race showed no fermentative characteristics different from the normal. This variation seems to be of the same general type as that previously described by me[26] as occurring in *Bacterium coli*, where long chains, isolated, gave rise to races morphologically different from the parent stock.

Strains of dysentery which present new morphological characteristics have been described by several authors, the new strains proceeding from aberrant colonies rather than from selected aberrant cells. Kruse, Rittershaus, Kemp, and Metz[27] found secondary colonies in dysentery and pseudodysentery cultures, which, when transplanted to bouillon, showed a growth having a tendency to clump. Baerthlein[28] has described morphologically aberrant colonies not only in dysentery but in cultures of cholera vibrios and of typhoid, paratyphoid, and Gaertner's bacilli. In both the Shiga-Kruse and the toxin-free types of dysentery bacilli, he obtained colonies which differ in form of colony, morphology of bacilli, and in agglutinability from the parent types.[29]

[26] *Loc. cit.*
[27] *Zeitschr. f. Hyg. u. Infectionskrankh.* (1907), **57**, 418.
[28] *Centralbl. f. Bakt. etc.*, Ref. (1911), **50**, Beiheft, 128.
[29] Baerthlein, *Arbeit. a. d. kais. Gesundheitsamte* (1912), **40**, 433.

DISCUSSION

As stated above, variations in the fermentative characteristics of cultures have been observed by many writers. By most authors this phenomenon has been regarded as a mutation such as De Vries observed in higher plants, in that the new races seem to appear suddenly and in a relatively small number of individuals and the new characteristics are constantly transmissible. Pringsheim,[30] however, regards a new characteristic such as the faculty of fermenting a given sugar as an adaptation to a new environment simply. Since sex and body cells are not separated in bacteria as in higher organisms, it is theoretically possible that such a new environment may impress upon the cells a characteristic which persists in the absence of the special stimulus. Such adaptive variations are not mutations in the sense of De Vries. Benecke [31] holds similar views regarding the adaptive character of variations of the *Bacterium coli mutabile* type. Burri,[32] after extensive experiments on *Bacterium coli mutabile* and *B. coli imperfectum*, holds that the new fermentative characteristic may be exhibited by the descendants of all individuals of the parent stem, while in the mutations of De Vries they are present in only from 1 to 3 per cent. Further, he concludes from his experiments that the characteristic is, in strict sense, not new, but a latent characteristic become active. It is gradually acquired, while the De Vries mutation arises suddenly and fully formed. Josef Klein,[33] working with *Bacterium coli mutabile*, confirms the work of Burri and regards these variations as adaptations gradually acquired rather than mutations in the ordinary use of the term. Baerthlein [34] holds that the above-described sudden appearance of new characteristics in strains of bacteria are true mutations. Such mutations, often indistinguishable from atavisms, have usually occurred in his experiments as the result of a change from a less favorable medium, such as is found in old agar or bouillon cultures, or in fæces, to a more favorable medium, as fresh agar. In other cases, the addition of various substances to the medium and the growth at high temperatures or the multiplication in the body of an infected animal seem to favor the production of mutations

[30] *Med. Klinik* (1911), 7, 144. Also, Die Variabilität niederer Organismen. Berlin (1910).

[31] *Zeitschr. f. induct. Abstammungs- u. Vererbungslehre* (1909), 2, 215.

[32] *Centralbl. f. Bakt. etc.*, II Abt. (1910), 28, 321.

[33] *Zeitschr. f. Hyg. u. Infektionskrankh.* (1912), 73, 87.

[34] *Arb. a. d. kais. Gesundheitsamte* (1912), 40, 433.

in lower organisms. In all cases, we may conceive that, whatever the stimulus, the mutation consists in the change of latent hereditary units (Progenen) to active ones (Genen), whether the change appears as an advance or an atavism.

The maltose-fermenting race of dysentery bacilli isolated by me was obtained from ordinary agar cultures, not from secondary colonies on maltose. The appearance of the characteristic on a maltose-free medium would point to a variation of a nonadaptive character, and would indicate that we have to do with a parent race originally non-stable with regard to maltose fermentation. The new characteristic is fully formed at the start, and persists through many generations; but the comparatively large percentage of acid-forming cells in the first series and the apparent instability of the parent culture might exclude this variation from the catagory of mutation. It is true that any cell of the non-maltose fermenting type may originate an acid-producing race; but many generations may intervene before the maltose-fermenting cells arise. The final decision in this matter must depend upon the definition of the term mutation.

With regard to the other form of variation described here, that appearing in strain 42, we certainly approach more nearly to the characteristics of a true mutation. The variation certainly appears suddenly and fully formed. It appears in a relatively small number of individuals, it is not adaptive, and the new characteristics are transmissible to offspring through many generations. This new race is to be compared with the one described by me in *Bacterium coli*, in which the selection of certain long threads gave rise to races permanently different from the parent stock. There is the possibility of regarding both cases as degenerations, but after they became started both types showed as much vegetative vigor as the parent race, while still retaining new characteristics. Whether within the strict scope of the definition of mutation or not, both cases seem comparable to sports appearing vegetatively on higher plants and capable of indefinite propagation. Some of these variations may at first show less vegetative vigor than the parent and be none the less regarded as true sports.

SUMMARY

1. From a culture of *Bacillus dysenteriæ*, Flexner type, derived from a single cell, 3 series of single-cell isolations were made at intervals of about five months. The first series gave 5 maltose-fermenting variants out of 21 isolations; the second, 5 out of 60;

the third, 1 out of 123. The other single-cell cultures as well as the parent culture render maltose alkaline.

2. The nonfermenting type produces secondary colonies consisting of normal and involution cells, either of which may develop acid- or alkaline-producing cultures. An ordinary transfer from a secondary colony, including many cells of both sorts, gives an acid-forming culture.

3. Selection from the acid-producing type failed to produce any but similar types, and selection from the alkaline-producing type gave only alkaline, provided secondary colonies were not chosen.

4. Mixed cultures, consisting of an equal number of cells of each type, showed that the two types may exist side by side through from 10 to 15 daily transfers, but with a tendency for the acid to outstrip the alkaline.

5. Transfer in maltose broth gave no increase in the acid-producing power except in old cultures.

6. Growth in various substances other than maltose failed to alter materially the characteristics of the two types.

7. In a specific serum, the two types showed approximately the same agglutination.

8. A permanent new race, characterized by morphological peculiarities, was obtained by the selection of an aberrant cell from a culture of dysentery of the Shiga-Kruse type.

Since the special technique used in this research has been described in other publications,[35] only the portion having to do with the making of one-cell cultures in series will be given here. A large cover glass (about 38 by 65 millimeters) is carefully cleaned, sterilized, and placed on the isolation chamber in the usual manner. Lines are ruled on the upper side with India ink or wax pencil, as shown in the illustration. Then, with a sterile pipette, bent at the tip, droplets of sterile broth about 2 millimeters in diameter are made on the undersurface of the cover, with a long drop *a* toward the open end of the box and a second large drop *b* near the other end.

Into a large drop *a* a small portion of actively growing culture is transferred by a loop or pipette. The chamber is now placed on the stage of the microscope, a fine-pointed pipette of the type used in isolation adjusted, and a considerable number of bacilli in medium dilution taken into the pipette from *a*. The

[35] See reference, under note 1. A complete description of the pipette technique and its various applications is in preparation, and will be published in a subsequent number of this Journal.

rows of hanging drops are now brought into the field, and a small droplet ejected from the pipette in the immediate neighborhood of each (fig. 1). Each smaller droplet must be made to contain a single bacillus, and, if a proper dilution has been used, this is easily done. The small droplets must be made very small so as to be easily examined and the presence of a single cell determined. If the first droplet contains no bacilli, others may be made near it at the margin of the larger drop until one is obtained as desired. If two or more bacilli come out, all but one may be picked up and carried to the next large drop.

When as many as desired of the larger drops are supplied with the one-bacillus droplets, the pipette is withdrawn and a new sterile one introduced.

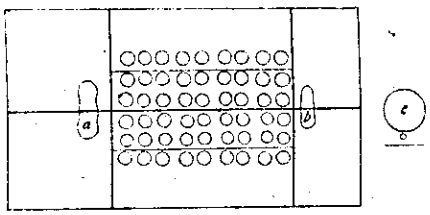


FIG. 1. Diagram of a microscopical slide to illustrate the method of single-cell isolation. *c* Larger droplet shown on larger scale with smaller droplet near it.

This may be filled with sterile broth from drop *b* or from a test tube. With this pipette, broth is applied to each small droplet until, with its contained bacillus, it merges with the larger drop, or, broth is supplied to the larger drop until it overflows the smaller one.

The larger drop affords sufficient broth for good growth, and the purpose of the smaller droplet is, obviously, to permit of thorough examination in order to be sure of the presence of a single cell.

The cover is now removed from the box and sealed over a shallow moist chamber. The next day, transfers to test tubes may be made from each drop with a bent capillary pipette that is made new for each drop or by means of a fine platinum wire bent at the tip.

By this method one can quickly obtain a series of pure cultures from one source. If plump, actively growing cells are selected, practically all will grow. It is best to use a young culture grown two or three hours in the same broth as that used in the drops. Agar may be used in place of the broth in making the rows of drops. Isolations from two or more sources may be

made on one cover, but it is usually best to employ separate covers for each. The nature of the growth in each drop may be examined microscopically before transfer, and a record kept by lettering and numbering the rows.

ADDENDUM

A special experiment was arranged to test the constancy of several different races of dysentery bacilli. All were isolated from stools of cases of dysentery occurring in Manila during the summer of 1912. There were 8 strains, 5 of them of the Shiga-Kruse type, 2 of the Flexner, and 1 with the characteristics of the Flexner but with a tendency to ferment lactose. Soon after isolation from the stools, a one-cell pure culture was made of each. This pure culture gave the same reactions as the stock on lactose-, glucose-, maltose-, and mannite-litmus agars. The cultures were now placed at room temperature on ordinary agar and transferred at about monthly intervals for about eleven months. At the end of this period fresh agar cultures were transferred to plain broth, glucose-broth fermentation tubes, litmus-lactose broth fermentation tubes, and to the following litmus agars: lactose, glucose, mannite, saccharose, maltose, levulose, dextrin, salacin, glycerine, erythrite, inulin, raffinose, galactose, amygdalin, and dulcite. The results of these cultures were the same as those observed eleven months before, and the one-cell pure culture gave the same reactions as the stock in every case. Apparently both the one-cell culture and the stock culture isolated from a colony had kept their characteristics constant during this period.

Well-marked secondary colonies were formed by 4 different strains, all of the Shiga-Kruse type. Two of these strains formed the secondary colonies only on saccharose-litmus agar, 1 on both saccharose and maltose, and 1 on saccharose and lactose. All secondary colonies were transferred to new tubes of litmus agar containing the appropriate sugar. All transfers from secondary colonies on saccharose gave acid-forming cultures, while those from the maltose and lactose gave alkali-forming cultures.

A test was made of the composition of the two strains which had produced secondary colonies only on saccharose. From a series kept on ordinary agar and never passed through saccharose, 45 single cells were isolated from one strain and 49 from the other, and the cultures from these 94 single cells were tested on saccharose-litmus agar. Only one culture proved to

be an acid-former. This culture lost its power of fermenting saccharose after several transfers on ordinary agar, and an acid-forming culture on saccharose also failed to form acid in subcultures. In order to test the composition of this originally saccharose-fermenting strain, 30 single cells were isolated from a culture which had been carried through several transfers on ordinary agar and which had never been grown on saccharose. None of the 30 cultures obtained from these cells formed acid on saccharose agar. Apparently the saccharose-fermenting variations of this strain of dysentery bacilli are less permanent than the maltose-fermenting variations of Flexner strain 105200.

ILLUSTRATIONS

PLATE I.

FIG. 1. Parent culture of *Bacillus dysenteriæ*, Shiga-Kruse type, strain No. 42.

2. A new race grown from a single cell isolated from the culture shown in fig. 1. Both are from 24-hour agar cultures grown under like conditions. Both had been grown about three days since the isolation of the new race, and had been passed through two agar cultures.

TEXT FIGURE

FIG. 1. Diagram of a microscopical slide to illustrate the method of single-cell isolation.

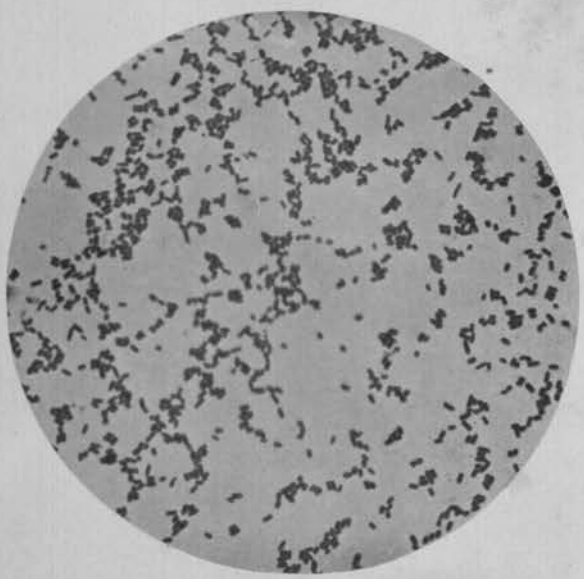


Fig. 1. Parent culture of Bacillus dysenteriæ, Shiga-Kruse type, strain No. 42.

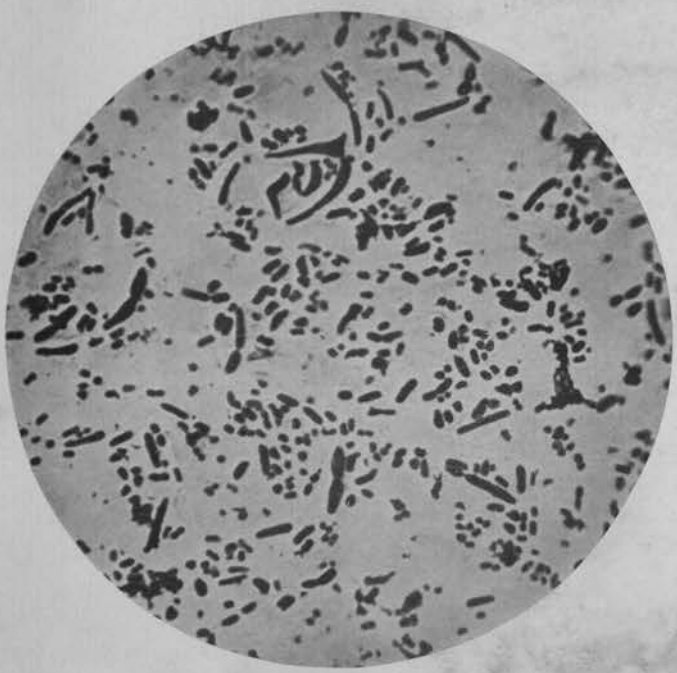


Fig. 2. A new race grown from a single cell isolated from the culture shown in fig. 1.

PLATE I.

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